

Lipoteichoic acid from *Staphylococcus aureus* enhances allergen-specific immunoglobulin E production in mice

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Summary

Background Our previous study demonstrated that lipoteichoic acid (LTA) from *Staphylococcus aureus* induced T helper type 2 (Th2)-prone dermatitis resembling that seen in atopic dermatitis (AD) patients in mice sensitized percutaneously with an allergen. However, the effects of LTA on allergen-specific IgE production in such sensitized mice have not been elucidated.

Objective The purpose of this study was to determine the effects of LTA from *S. aureus* on allergen-specific IgE production in mice sensitized percutaneously with a house dust mite antigen (MA).

Methods Mice were sensitized with a single topical application of MA and/or LTA to barrier-disrupted abdominal skin. One to 5 weeks later, MA-specific IgE antibodies in sera from sensitized mice were detected by an enzyme-linked immunosorbent assay (ELISA). Expression of B7.1 (CD80), B7.2 (CD86) and CD40L molecules by CD40-positive (CD40⁺) and CD4-positive (CD4⁺) cells in the lymph nodes of sensitized mice were analysed by flow-cytometry (FACS).

Results Simultaneous sensitization with MA and LTA increased IgE production 3 weeks later, significantly more than sensitization with MA alone. FACS analysis of CD40⁺ cells in the lymph nodes from sensitized mice showed that simultaneous sensitization with MA and LTA did not enhance CD80- or CD86-expression by antigen-presenting cells such as B lymphocytes and dendritic cells more than sensitization with MA alone. However, analysis of CD4⁺ cells in the lymph nodes showed that simultaneous sensitization with MA and LTA increased the number of CD40L-expressing Th cells more than sensitization with MA alone.

Conclusion These results suggest that LTA enhances allergen-specific IgE production by a mechanism associated with up-regulation of CD40L-expressing Th cells and this might explain the role of skin colonization with *S. aureus* in AD patients.

Keywords atopic dermatitis, CD80, CD86, IgE, IL-4, lipoteichoic acid, *Staphylococcus aureus*

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease with immunopathologic features that vary depending on the duration of the lesion. Patients with AD show increased serum IgE levels (70–80% of patients), enhanced synthesis of IL-4 and IL-5 in their early skin lesions and *Staphylococcus aureus* infection [1–3]. IL-4 and IL-5 are known to induce the IgE switch of B cells and eosinophilia, respectively, and are thought to play important roles in the induction of acute and/or chronic inflammation in AD [1]. *S. aureus* can be isolated from 75–90% of lichenified plaques, whereas the skins of only 5% of healthy individuals are colonized by this organism [4].

Many studies have indicated that *S. aureus* amplifies skin inflammation in AD by secreting a group of toxins known to act as superantigens, which vigorously stimulate T cells [5–7].

However, some investigators have suggested that other pathogenic factors are also involved in AD, because superantigen production was found neither exclusively in AD patients nor in all AD patients [7–9]. In our previous study, the detection rates of superantigen-producing *S. aureus* on the skin lesions and lesion-free skin of AD patients did not differ significantly and we suggested that *S. aureus* possesses other, unidentified, pathogenic factors [10].

Gram-positive bacterial cell walls are composed of highly cross-linked peptidoglycan (PEG) decorated to a variable extent with teichoic acid polymers. The latter are also linked to plasma membrane phospholipids, of which lipoteichoic acid (LTA) and PEG are the major surface components [11, 12]. Gram-positive bacteria contain no lipopolysaccharide (LPS), and LTA and/or PEG are thought to be major inflammatory products in their cell walls. In previous studies, we found that LTA could induce IL-5 production by peripheral blood mononuclear cells (PBMCs) from patients with AD [13] and it induced AD-like inflammation and T helper type 2 (Th2) cytokine responses, including IL-4 and IL-5 production, in an allergen-independent manner in mice sensitized percutaneously with an allergen [14]. Recent

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studies have demonstrated that percutaneous sensitization of mice with allergens elicits Th2 cytokine responses like those seen in AD patients [14–18] and such mice have been used as an experimental model of AD. However, whether LTA affects allergen-specific IgE production in AD patients is not clear. In this study, therefore, we investigated the effects of LTA on allergen-specific IgE production in mice sensitized percutaneously by applying an allergen to barrier-disrupted skin as an experimental animal model of AD.

Materials and methods

Allergen, superantigen and LTA

Mite antigen (MA) was prepared from a commercially available house dust mite body extract (Torii Pharmaceutical Co. Ltd, Tokyo, Japan), which is a 50% glycerol saline extract of *Dermaphagoides farinae*. The extract was dialysed against phosphate-buffered saline (PBS), pH 7.4 and concentrated to 1 mg/mL with a Centricon 10 (Amicon Division of W.R. Grace & Co., Conn., Beverly, MA, USA) to produce the MA preparation. LTA and toxic shock syndrome toxin-1 (TSST-1) derived from *S. aureus* were obtained from Sigma Chemical Co. (St Louis, MO, USA) and Toxin Technology, Inc. (Sarasota, FL, USA), respectively.

Mice

Female specific-pathogen-free BALB/c mice were obtained from Tokyo Laboratory Animals Science Co. Ltd (Tokyo, Japan) and used when they were 6–8 weeks of age. They were housed in plastic cages in a clean, air-conditioned room at 24 °C and allowed free access to a standard laboratory diet and water.

Percutaneous sensitization

Percutaneous sensitization of mice with MA, LTA and TSST-1 was performed as described previously [14]. Briefly, the skin of each mouse was barrier-disrupted by repeatedly (eight times) applying adhesive cellophane tape to shaved abdominal skin then pulling it off. Then, 100 µL MA solution (1 mg/mL in PBS), 50 µL LTA solution (0.125–1 mg/mL in PBS) and 50 µL TSST-1 solution (0.5 mg/mL in PBS), respectively, were applied topically to a 10-cm² area of barrier-disrupted abdominal skin. LTA-, TSST-1- and MA-non-sensitized control mice were treated by applying 50 µL, 50 µL and 100 µL PBS, respectively, to the barrier-disrupted skin in the same manner.

Enzyme-linked immunosorbent assay

One to five weeks after sensitization, sera were collected from the mice and stored at –80 °C until analysis. Enzyme-linked immunosorbent assay (ELISA) microplates (H type plate; Sumitomo Bakelite Co. Ltd, Tokyo, Japan) were coated with 100 µL MA (20 µg/mL) in PBS for 2 h at 37 °C, after which the plates were washed with PBS-Tween 20 (0.05%), blocked with 25% Block Ace (Yukijirushi, Hokkaido, Japan) for 1 h at 37 °C and washed again with PBS-Tween 20. The serum samples were diluted 10-fold with 10% Block Ace, and 100-µL aliquots were added to the wells, which were incubated for 1 h at room temperature. After washing with PBS-Tween 20, 100 µL biotin-labelled rat anti-mouse IgE monoclonal antibody (2 µg/mL; clone R35-92; PharMingen, San Diego, CA, USA) in 10%

Block Ace was added to each well and incubated for 1 h at room temperature. After washing with PBS-Tween 20, 100 µL streptavidin-biotinylated horseradish peroxidase complex (Amersham Life Science, Arlington Heights, IL, USA) diluted 1 : 500 in 10% Block Ace was added to each well and incubated for 30 min at room temperature. Then, the plates were washed with PBS-Tween-20, 100 µL ABTS (3-ethylbenzthiazoline-6-sulphonic acid; Boehringer Mannheim GmbH, Mannheim, Germany) substrate buffer was added, the plates were incubated for 30 min at room temperature and the absorption at 405 nm of each well was read. Quantities of antigen-specific IgE were calculated by comparison with a reference curve generated with the use of a mouse anti-dinitrophenyl (DNP) monoclonal IgE antibody (Sigma Chemical Co.) and its antigen, DNP-conjugated human serum albumin (Sigma Chemical Co.), as described by Li et al. [19].

Flow cytometric analysis

Expression of B7.1 (CD80) and B7.2 (CD86) molecules on CD40-positive (CD40⁺) cells in the lymph nodes was monitored by measuring the immunofluorescence intensity after immunostaining with monoclonal antibodies. Murine lymph node cells (10⁶ cells/staining) were incubated on ice for 30 min with a fluorescein-conjugated rat monoclonal anti-mouse CD80 or CD86 antibody (clones RMMP-1 and RMMP-2, respectively; Cosmo Bio Co. Ltd, Tokyo, Japan). A R-phycoerythrin-conjugated rat monoclonal anti-mouse CD40 antibody (clone 3/23; Cosmo Bio Co. Ltd) was also used to detect antigen-presenting cells (APCs), such as mature B cells and dendritic cells, in the lymph nodes. Expression of the CD40 ligand (CD40L) on CD4⁺ T cells in the lymph nodes was also monitored by measuring the immunofluorescence intensity after immunostaining. Murine lymph node cells (10⁶ cells/staining) were incubated on ice for 30 min with a R-phycoerythrin-conjugated hamster monoclonal anti-mouse CD40L antibody (clone MR1; Southern Biotechnology Associates, Inc., Birmingham, AL, USA) and a fluorescein-conjugated rat monoclonal anti-mouse CD4 antibody (clone RM4-5; PharMingen). All cells were washed three times with PBS before analysis and the immunolabelled cell preparations were analysed using a FAC-Sort flow-cytometer (Becton Dickinson, San Jose, CA, USA).

Semiquantitative RT-PCR assay

In order to determine the IL-4 and IL-13 mRNA expression levels of lymph node cells, total RNA was extracted from these cells by the single-step method with TRI-Reagent (Molecular Research Center, Cincinnati, OH, USA). Then, cDNA was synthesized from 8 µg total RNA using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). The PCR was performed using the following primers: β-actin (540 bp) 5' primer, 5'-GTGGGCCGCTCTAG GCACCAA-3' and 3' primer, 5'-CTCTTTGATGTCACG CACGATTTC-3'; IL-4 (400 bp) 5' primer, 5'-AGTTGTCA TCCTGCTCTTCTTCTC-3' and 3' primer, 5'-CGAGTA ATCCATTTGCATGATGCTC-3'; IL-13 (335 bp) 5' primer, 5'-GCCGGGATGGGCATTCCACGTGTG-3' and 3' primer, 5'-GGACGCCAAGGTCAAGAACAGTTG-3'. Each PCR was performed using a GeneAmp PCR System 9700 (Perkin-Elmer Co., Norwalk, CT, USA) and the PCR products were

analysed by 2% agarose gel electrophoresis, as described previously [14].

Statistics

The data were expressed as mean $\pm$ SD and differences between means were analysed using Student's *t*-test with a two-tailed test of significance. Differences at $P < 0.05$ were considered to be significant.

Results

Effects of LTA on allergen-specific IgE production

The first experiments were carried out to examine the production of allergen-specific IgE after percutaneous sensitization of mice with MA. Mice were sensitized by applying 100 μ g MA to a 10-cm² area of barrier-disrupted abdominal skin and, 1–5 weeks later, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. Figure 1 shows the time course of allergen-specific IgE production in mice sensitized percutaneously with MA. The MA-specific IgE level was significantly higher than that of the control mice 2 weeks after sensitization, peaked at 3 weeks and declined thereafter. These results indicate that percutaneous sensitization with MA induced allergen-specific IgE production in mice.

Next, the influence of LTA on allergen-specific IgE production induced by percutaneous sensitization with MA was examined. Mice were sensitized by applying MA (100 μ g/10 cm²) and LTA (6.25–50 μ g/10 cm²) simultaneously to barrier-disrupted abdominal skin and, 3 weeks later, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. Figure 2 shows that simultaneous sensitization with MA and LTA enhanced allergen-specific IgE production more than sensitization with MA alone. The enhancement of allergen-specific IgE production was significant after the application of 12.5 μ g LTA and peaked after the application of more than 25 μ g LTA. These results indicate that percutaneous sensitization with LTA up-regulated allergen-specific IgE production in MA-sensitized mice. This capability to enhance allergen-specific IgE production was specific to LTA and was not accepted by LPS (data not shown). Figure 3 shows the influence

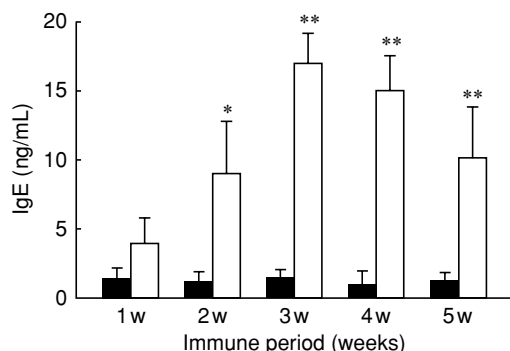


Fig. 1. Time course of allergen-specific IgE production after percutaneous sensitization with MA. Mice were treated with 100 μ L PBS (■) or sensitized with 100 μ L 1 mg/mL MA solution (□) applied to a 10-cm² area of barrier-disrupted abdominal skin. One to 5 weeks later, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. The results are expressed as mean $\pm$ SD ($n = 5$). * $P < 0.05$; ** $P < 0.005$ cf. PBS treatment.

of the timing of the LTA challenge on the enhancement of allergen-specific IgE production in MA-sensitized mice. When mice were sensitized with MA (100 μ g/10 cm²) and challenged with LTA (25 μ g/10 cm²) 1 week later, the increases in their serum IgE levels were similar to those of mice sensitized simultaneously with MA and LTA. However, when mice were challenged with LTA 1 week before sensitization with MA, no increases in their serum IgE levels over those seen with MA sensitization alone were observed. Figure 4 shows the influence of simultaneous challenge with LTA and TSST-1 on the enhancement of allergen-specific IgE production in MA-sensitized mice. When mice were sensitized simultaneously

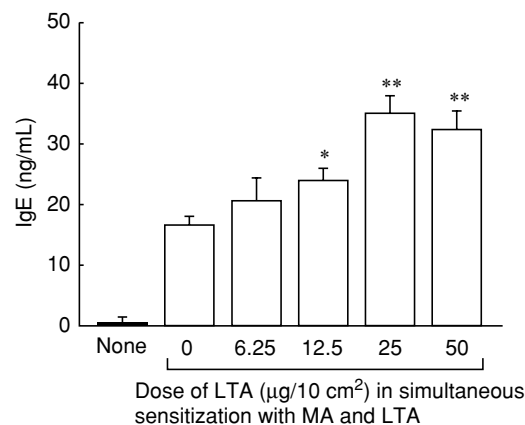


Fig. 2. Enhancement of allergen-specific IgE production after percutaneous sensitization with LTA and MA. Mice were sensitized with 100 μ L MA solution (1 mg/mL) and 50 μ L LTA solution (0.125–1 mg/mL) applied simultaneously to a 10-cm² area of barrier-disrupted abdominal skin. LTA- and MA-non-sensitized control mice were treated by applying 50 μ L PBS and 100 μ L PBS, respectively, to barrier-disrupted skin in the same manner. Three weeks after sensitization, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. The results are expressed as mean $\pm$ SD ($n = 5$). * $P < 0.05$; ** $P < 0.005$ cf. LTA-non-sensitized mice.

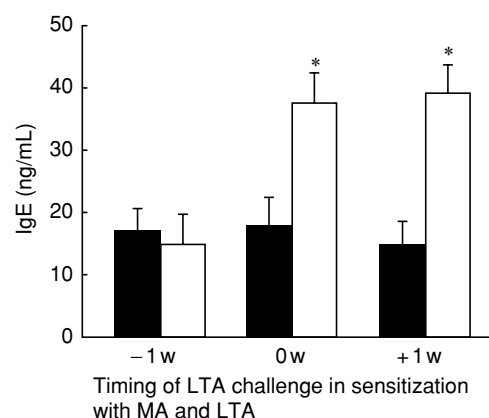


Fig. 3. Enhancement of allergen-specific IgE production by LTA and the influence of the timing of the LTA challenge. Mice were sensitized by applying 100 μ L MA solution (1 mg/mL) and 50 μ L LTA solution (0.5 mg/mL) to a 10-cm² area of barrier-disrupted abdominal skin. LTA was applied 1 week before (–1w), at the same time as (0w) or 1 week after (+1w) sensitization with MA (□). LTA-non-sensitized mice (■) were treated by applying 50 μ L PBS instead of LTA solution to the barrier-disrupted skin and sensitized with MA, as described above. Three weeks after sensitization with MA, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. The results are expressed as mean $\pm$ SD ($n = 5$). * $P < 0.005$ cf. LTA-non-sensitized mice.

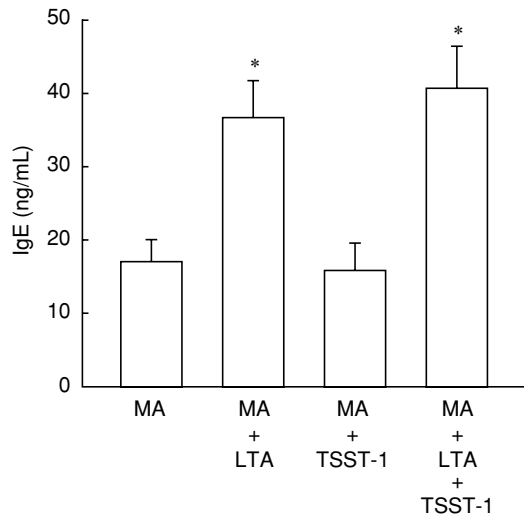


Fig. 4. Influence of simultaneous challenge with LTA and TSST-1 on the enhancement of allergen-specific IgE production. Mice were sensitized by applying 100 μ L MA solution (1 mg/mL), 50 μ L LTA solution (0.5 mg/mL) and 50 μ L TSST-1 solution (0.5 mg/mL) simultaneously to a 10-cm² area of barrier-disrupted abdominal skin. LTA- and TSST-1-non-sensitized mice were treated by applying each 50 μ L PBS to the barrier-disrupted skin in the same manner. Three weeks after sensitization, sera were collected and processed for the detection of allergen-specific IgE antibodies by an ELISA. The results are expressed as mean $\pm$ SD ($n=5$). * $P < 0.01$ cf. LTA- and TSST-1-non-sensitized mice.

with MA (100 μ g/10 cm²) and TSST-1 (25 μ g/10 cm²), the increases in their serum IgE levels like those seen in the simultaneous sensitization with MA (100 μ g/10 cm²) and LTA (25 μ g/10 cm²) were not observed. Furthermore, the serum IgE levels were no higher after simultaneous sensitization with MA, LTA and TSST-1 than after simultaneous sensitization with MA and LTA.

Effects of LTA on CD80, CD86, CD40L and IL-4 mRNA expression by lymph node cells

In order to elucidate the mechanisms responsible for the enhancement of allergen-specific IgE production by LTA, the effects of LTA on the expression of CD80 and CD86 by APCs (CD40⁺ cells) and of CD40L by Th cells (CD4⁺ cells) were studied using lymph node cells from sensitized mice. Mice were sensitized by applying MA (100 μ g/10 cm²) alone or together with LTA (25 μ g/10 cm²) to barrier-disrupted abdominal skin. Three weeks later, lymph node cells were obtained from the non-sensitized and sensitized mice, stained with an anti-CD80 or anti-CD86 monoclonal antibody in combination with an anti-CD40 monoclonal antibody to detect B7.1 (CD80) or B7.2 (CD86) molecules on the surfaces of the CD40⁺ cells and analysed by flow-cytometry. As shown in Fig. 5, spontaneous CD80 expression was observed in CD40⁺ cells of non-sensitized mice (17.3%) and the expression level was not influenced by sensitization with MA alone or with LTA. In contrast, CD86 expression by CD40⁺ cells in the lymph nodes of MA-sensitized mice increased slightly compared with non-sensitized mice (from 3.6 to 7.5%), although simultaneous sensitization with MA and LTA did not enhance CD86 expression further.

Next, the same lymph node cells were stained with an anti-CD40L monoclonal antibody in combination with an anti-CD4 monoclonal antibody to detect CD40L molecules on the CD4⁺

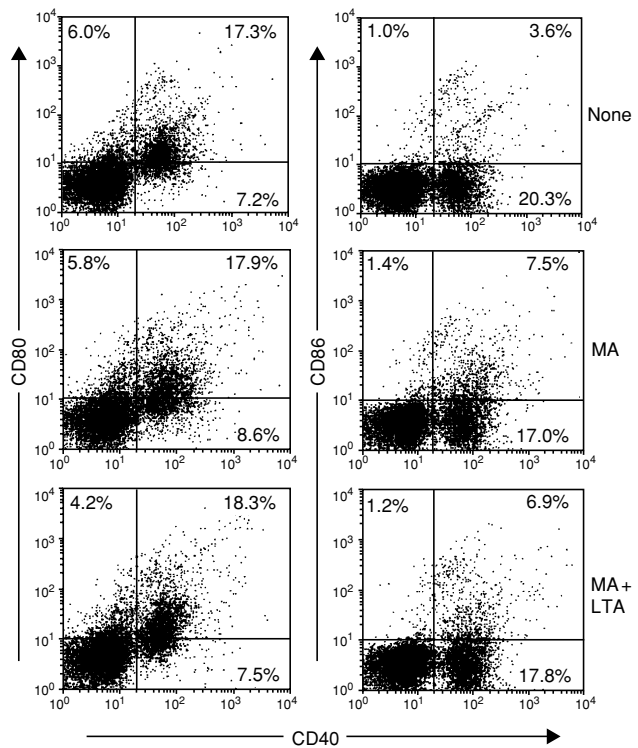


Fig. 5. Effects of LTA on CD80 and CD86 expression by CD40⁺ cells in the lymph nodes. Mice were sensitized by applying 100 μ L MA solution (1 mg/mL) and 50 μ L LTA solution (0.5 mg/mL) simultaneously to a 10-cm² area of barrier-disrupted abdominal skin. LTA- and MA-non-sensitized control mice were treated by applying 50 μ L PBS and 100 μ L PBS, respectively, to the barrier-disrupted skin in the same manner. Three weeks after sensitization, lymph node cells were obtained from the non-sensitized and sensitized mice, stained with an anti-CD80 or CD86 monoclonal antibody in combination with an anti-CD40 monoclonal antibody to detect B7.1 (CD80) and B7.2 (CD86) molecules on the CD40⁺ cell surfaces and analysed by flow-cytometry. The data shown are representative results of four independent experiments.

cell surfaces and analysed by flow-cytometry. As shown in Fig. 6, in comparison with non-sensitized mice, CD40L expression on the CD4⁺ cell surfaces increased after sensitization with MA (from 1.6 to 19.6%) and simultaneous sensitization with MA and LTA increased the number of CD4⁺ cells that expressed CD40L (31.7%) compared with sensitization with MA alone (19.6%).

Subsequently, in order to determine whether the same lymph node cells from sensitized mice could still produce IL-4 or IL-13, 3 weeks after sensitization, IL-4 and IL-13 mRNA expression in the lymph node cells were confirmed by semiquantitative RT-PCR analysis. Figure 7 shows that the lymph node cells from MA-sensitized mice expressed IL-4 mRNA, but not IL-13 mRNA, 3 weeks after sensitization. However, the IL-4 mRNA expression levels were no higher after simultaneous sensitization with MA and LTA than after MA alone, and LTA itself was unable to induce IL-4 mRNA expression.

Discussion

A high incidence of chronic skin colonization with *S. aureus* is a prominent feature of AD. Although many attempts have been made to characterize the role of *S. aureus* on the skin of AD

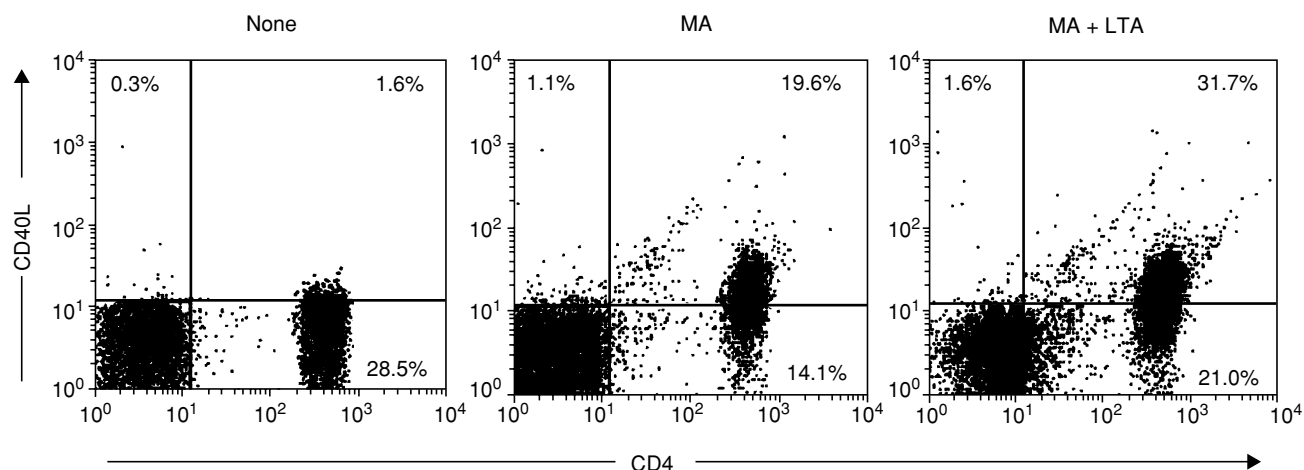


Fig. 6. Effect of LTA on CD40L expression by CD4⁺ cells in the lymph nodes. Mice were sensitized by applying 100 μ L MA solution (1 mg/mL) and 50 μ L LTA solution (0.5 mg/mL) simultaneously to a 10-cm² area of barrier-disrupted abdominal skin. LTA- and MA-non-sensitized control mice were treated by applying 50 μ L PBS and 100 μ L PBS, respectively, to the barrier-disrupted skin in the same manner. Three weeks after sensitization, lymph node cells were obtained from the non-sensitized and sensitized mice, stained with an anti-CD40L monoclonal antibody in combination with an anti-CD4 monoclonal antibody to detect CD40L molecules on the CD4⁺ cell surfaces and analysed by flow-cytometry. The data shown are representative results of four independent experiments.

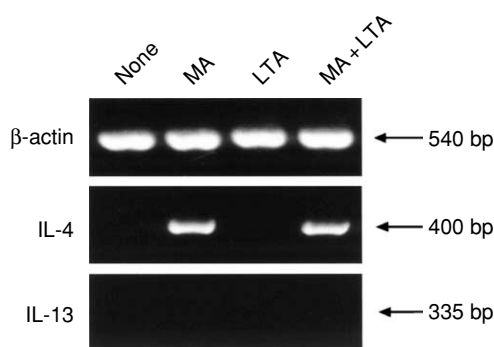


Fig. 7. Effect of LTA on IL-4 mRNA expression in lymph node cells. Mice were sensitized by applying 100 μ L MA solution (1 mg/mL) and 50 μ L LTA solution (0.5 mg/mL) simultaneously to a 10-cm² area of barrier-disrupted abdominal skin. LTA- and MA-non-sensitized mice were treated by applying 50 μ L PBS and 100 μ L PBS, respectively, to the barrier-disrupted skin in the same manner. Three weeks after sensitization, cytoplasmic RNA was extracted from the lymph node cells of the non-sensitized and sensitized mice, reverse-transcribed and amplified by the PCR using β -actin, IL-4 and IL-13 primer sets. The data shown are representative results of four independent experiments.

patients, most studies have focused on the role of bacterial superantigens in AD. Some studies demonstrated that staphylococcal enterotoxins act as superantigens and increase the release of the Th2 cytokines IL-4 and IL-5 by PBMCs from patients with AD [6]. These cytokines are known to induce the IgE switch of B cells and eosinophilia, which is often observed in AD patients [1]. Finally, 80% of AD patients exhibit elevated serum IgE levels. However, half of the *S. aureus* strains isolated from AD patients are not capable of producing superantigens [7, 8, 10] and therefore the roles of these strains in the skin lesions of AD patients are not fully understood.

The purpose of this study was to determine whether allergen-specific IgE production is enhanced by LTA, which is a major component of the cell wall of *S. aureus*. Our data showed that LTA enhanced allergen-specific IgE production in mice sensitized percutaneously with MA. This enhancement was observed

when mice were challenged with LTA 1 week after or at the same time as MA sensitization. IgE production induced by MA-sensitization involved increased B7.2 (CD86)-expression by CD40⁺ cells in the lymph nodes. Jirapongsananuruk et al. [20] demonstrated abnormally high B7.2 expression by B cells from patients with AD, suggesting a potential role for B7.2 in the induction of IgE synthesis. Therefore, the enhancement of B7.2 expression on B cells appears to play an important role, at least in AD. The low B7.2 expression levels after MA-sensitization we observed may be attributable to our experimental conditions in which mice were sensitized with a limited dose of a specific allergen. Repeated sensitization with the allergen MA may increase B7.2 expression corresponding to the increase in allergen-specific IgE production. However, the enhancement of IgE production induced by LTA was not associated with a further increase in B7.2-expression by CD40⁺ cells.

Next, we showed that IgE production induced by MA-sensitization involved increased CD40L-expression by CD4⁺ cells and IL-4 mRNA expression levels in the lymph nodes. B cells require two signals for induction of IgE synthesis. The first signal is delivered by the cytokines IL-4 or IL-13 and results in ϵ germ line transcription [21–23]. The second is delivered as a result of the interaction between the B cell antigen CD40 and its ligand CD40L, which is expressed by T cells in response to activation via their antigen receptors [24, 25], and leads to IgE switching, expression of mature ϵ RNA and IgE synthesis. In this study, we observed that the IL-4 mRNA expression levels in lymph nodes from mice sensitized with MA alone and with both MA and LTA were the same. However, sensitization with both MA and LTA resulted in more CD40L-expressing CD4⁺ cells than sensitization with MA alone. Therefore, our results indicate that the increase in allergen-specific IgE production induced by LTA depended on the induction of up-regulation of CD40L-expressing Th cells, which in turn augmented isotype switching in B cells by the CD40L–CD40 interaction in the presence of IL-4.

The lack of effect of prior sensitization with LTA on the increase in MA-specific IgE production suggests that LTA

only affects T cells that have been activated by MA-sensitization. Previous reports suggested that percutaneous sensitization of mice with MA would activate T cells, shift toward a Th2-prone cytokine profile and contribute to IgE production [14,16]. Therefore, the enhancement of B7.2 expression in CD40⁺ cells and CD40L expression in CD4⁺ cells would be a consequence of an interaction (antigen presentation vs. antigen recognition) between CD40⁺ B cells and the antigen-specific Th2 cells induced as a result of MA-sensitization. The induction of up-regulation of CD40L-expressing Th cells by LTA may result from activation of APCs such as Langerhans cells (LCs) in the epidermis. Probably, LCs would be stimulated by LTA through Toll-like receptor (TLR) 2 [26], and direct cell-to-cell contact between the activated LCs and the antigen-specific Th2 cells would provide a further signal for the antigen-specific Th2 cells. Re et al. [27] suggest that TLR2 stimulation may inhibit Th1 responses through the release of the IL-12 inhibitory p40 homodimer. As a result, therefore, Th2 cells may predominate, enhance more CD40L expression and induce more B cells to switch to IgE, with a subsequent overproduction of allergen-specific IgE. The lack of effect of sensitization with LPS, a TLR4 agonist or TSST-1, a superantigen on the increase in MA-specific IgE production would depend on the lack of a signal through TLR2. Otherwise, percutaneous invasion of at least TSST-1 may be unable to stimulate directly T cells in the lymph nodes because of antigen uptake and antigen processing by epidermal LCs, even though TSST-1 induces CD40L expression in T cells *in vitro* and causes IgE isotype switching in B cells in a CD40L-mediated mechanism [28]. Therefore, our findings suggest that percutaneous invasion of LTA, derived from skin-colonizing *S. aureus* would be superior to that of superantigens in the polyclonal increase in allergen-specific IgE production in AD patients.

Previous reports have not explained the role of non-toxin-producing strains of *S. aureus* in allergen-specific IgE production in AD patients. Our present results suggest that *S. aureus* strains that do not produce toxins that act as superantigens may play a critical role in perpetuating skin tissue inflammation through IgE production enhanced by LTA. As the skins of most AD patients show superficial *S. aureus* colonization and barrier disruption due to reduced ceramide levels [29], LTA would be expected to penetrate their skins. Therefore, antimicrobial treatment in at least a subgroup of AD patients, irrespective of whether they show clinical signs of superinfection, should be considered as a new therapeutic strategy for AD.

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