

ORIGINAL ARTICLE

Peptidoglycan-induced T helper 2 immune response in mice involves interleukin-10 secretion from Langerhans cells

Katsuhiko Matsui and Akemi Nishikawa

Department of Immunobiology, Meiji Pharmaceutical University, Tokyo, Japan

ABSTRACT

Patients with atopic dermatitis (AD) have superficial skin colonization with *Staphylococcus aureus* and an increased number of T helper (Th)2 cells in their peripheral blood. The purpose of this study was to clarify the involvement of interleukin (IL)-10 secretion from Langerhans cells (LCs) in staphylococcal peptidoglycan (PEG)-induced Th2 immune responses in mice. Mice were primed with LCs pulsed with PEG (or LPS) and ovalbumin (OVA) and then given a booster OVA injection 2 days later in the hind footpad. Five days after the OVA injection, cytokine responses in the draining popliteal lymph nodes were investigated by RT-PCR and ELISA. Production of both IL-10 and IL-12 by cultured LCs was detected by ELISA. Administration of PEG- or LPS-stimulated LCs into the hind footpads of the mice induced Th2-prone and Th1-prone immune responses, respectively, as represented by expression of IL-4 and interferon- γ . *In vitro* experiments showed that PEG induced greater production of IL-12 p40 from LCs than did LPS, whereas LPS induced greater production of IL-12 p70 from LCs than did PEG. Furthermore, it was found that PEG-stimulated LCs induced greater production of IL-10 than did LPS-stimulated LCs, and that neutralization of IL-10 augmented IL-12 p70 production and inhibited Th2 development by PEG-stimulated LCs. These results suggest that PEG can induce Th2 development through down-regulation of IL-12 p70 production by LCs in an IL-10 production-dependent manner and would explain the role of *S. aureus* colonization in patients with AD.

Key words Langerhans cells, peptidoglycan, *Staphylococcus aureus*, T helper 2 immune response.

Atopic dermatitis is a chronic inflammatory skin disease with immunopathologic features that vary according to the duration of the lesions. Most AD patients have superficial skin colonization by *Staphylococcus aureus* and increased expression of Th2 cytokines such as IL-4, IL-5 and IL-13 in their peripheral blood mononuclear cells (1). *S. aureus* can be isolated from 96–100% of skin lesions of AD patients, whereas the skin of only 0–10% of healthy individuals is colonized by this organism (2, 3). We have also found that *S. aureus* is more frequently detected in lesioned than in non-lesioned skin of AD patients and that the *S. aureus* bacterial cell count in lesioned skin is significantly higher than that in non-lesioned skin (3). However, there is no

significant difference between lesioned and non-lesioned skin of AD patients in the rate of detection of superantigenic exotoxin produced by *S. aureus*.

Gram-positive bacterial cell walls are composed of highly cross-linked PEG decorated to a variable extent with teichoic acid polymers. The latter are also linked to plasma membrane phospholipids, forming LTA, which is another major cell wall component (4, 5). Gram-positive bacteria contain no LPS; LTA and/or PEG are thought to be the major inflammatory products in their cell walls. Therefore, we postulated that LTA and/or PEG from *S. aureus* may play a more important pathogenic role than superantigenic exotoxins in AD patients. In previous studies, we found that LTA and PEG induces IL-5

Correspondence

Katsuhiko Matsui, Department of Immunobiology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan.
Tel: +81 424 95 8741; fax: +81 424 95 8612; email: kmatsui@my-pharm.ac.jp

Received 30 July 2012; revised 17 October 2012; accepted 11 November 2012.

List of Abbreviations: AD, atopic dermatitis; DC, dendritic cell; *E. coli*, *Escherichia coli*; IFN, interferon; IL, interleukin; LCs, Langerhans cells; LPS, lipopolysaccharide; LTA, lipoteichoic acid; OVA, ovalbumin; PEG, peptidoglycan; *S. aureus*, *Staphylococcus aureus*; Th, T helper; TLR, Toll-like receptor.

production by peripheral blood mononuclear cells from patients with AD (6) and that intradermal injection of LTA induces localized AD-like inflammation associated with significantly increased numbers of Th2-type cells in the dermis of allergen-sensitized mice (7). Furthermore, our recent studies have demonstrated that percutaneous injection of PEG from *S. aureus* induces Th2 cell infiltration in the dermis (8) and that PEG can induce a systemic Th2-dominant immune response like that seen in AD patients (9). However, the mechanisms responsible for induction of a systemic Th2 immune response by PEG are still insufficiently clear.

Langerhans cells are bone marrow-derived major histocompatibility complex class II positive antigen-presenting cells localized in the epidermis and mucosa. They are of DC lineage and essential for primary and secondary T cell-dependent immune responses (10). DCs are crucial in determining the outcomes of antigen encounters and integrating signals derived from antigens, the inflammatory context and the host environment into signals that can be read by naïve T cells in lymphoid tissues and by effector T cells in peripheral tissues. LCs can secrete IL-12 (11), which is produced predominantly by DCs and macrophages (12, 13). Bioactive IL-12, IL-12 p70, is a heterodimeric cytokine consisting of two disulfide-linked subunits, p35 and p40 (14). This cytokine is known to activate T cells in multiple ways and to increase production of cytokines such as IFN- γ , thereby serving as a powerful mediator of Th1-type differentiation both *in vitro* and *in vivo* (15). Monomers of the p40 and p35 subunits themselves do not possess IL-12 activity, but the homodimer of p40 has been shown to bind to the IL-12 receptor and work as an IL-12 p70 antagonist (16–18). In the present study, therefore, we investigated Th1/Th2 regulation of murine LCs stimulated with PEG or LPS and the involvement of IL-10 secretion by LCs.

MATERIALS AND METHODS

Peptidoglycan and lipopolysaccharide

Peptidoglycan derived from *S. aureus* was obtained from Fluka (Buchs, St Gallen, Switzerland), reconstituted in PBS, pH 7.4, at a concentration of 1 mg/mL, and sonicated for 1 hr before use. Highly purified LPS derived from *E. coli* R515 (Re) (1 mg/mL) was obtained from Alexis Biochemicals (Nottingham, UK). In all the present experiments, 10 μ g/mL PEG and 1 μ g/mL LPS were used as the optimal concentrations.

Mice

Specific-pathogen-free BALB/c (wild type) mice and DO 11.10 TCR Tg mice (OVA_{323–339}-specific I-A^d-restricted

TCR-transgenic mice) were obtained from Japan SLC (Hamamatsu, Japan) and the Jackson Laboratory (Bar Harbor, ME, USA), respectively, and used at the age of 6–8 weeks. They were housed in plastic cages with sterilized paper bedding in a clean, air-conditioned room at 24°C and allowed free access to a standard laboratory diet and water. All procedures performed on the mice were in accordance with the Guidelines of the Animal Care and Use Committee of Meiji Pharmaceutical University, Tokyo.

Purification of Langerhans cells

Langerhans cells in the epidermis were separated as described by Tada *et al.* (19). Briefly, BALB/c mouse skin was treated with dispase (3000 U/mL, Godo Shusei, Tokyo, Japan) in RPMI 1640 medium with L-glutamine (Sigma, St. Louis, MO, USA) containing 10% FBS (Sigma), 25 mM HEPES (Sigma), 100 U/mL penicillin and 100 μ g/mL streptomycin (Gibco RBL, Grand Island, NY, USA) (RPMI 10) for 3 hr at 37°C. The epidermis was separated from the dermis and incubated in RPMI 10 containing 0.025% deoxyribonuclease I (Sigma) for 20 min at room temperature. An epidermal cell suspension was obtained by vigorous pipetting of epidermal sheets. This suspension was then treated with mouse anti-mouse I-A^d monoclonal antibody (clone 040–220, IgG2a) (1:600; Meiji Dairies, Tokyo, Japan) in RPMI 10 for 45 min on ice. The cells were then incubated in plates precoated with goat anti-mouse IgG polyclonal antibody (1:100, Sigma) for 45 min at 4°C, and adherent cells were used as LCs. I-A^d positive LCs were purified to around 95% purity, as determined by flow cytometry.

Stimulation of LCs, immunization protocol, and preparation of lymph node cells

T helper 1/Th2 regulation with PEG or LPS was investigated according to the method of Maldonado-López *et al.* (20) with modifications. Briefly, LCs were adjusted to 5×10^5 cells/mL in RPMI 10, and then incubated with 30 μ g/mL OVA in the presence or absence of 0.1–10 μ g/mL PEG or LPS at 37°C in a humidified atmosphere with 5% CO₂. The cells were collected after incubation for 18 hr, washed in RPMI 10, and administered at a dose of 1×10^5 cells into both hind footpads of BALB/c mice. After 2 days, 30 μ g OVA was injected into both hind footpads of BALB/c mice as a booster. Draining popliteal lymph nodes were harvested from the popliteal fossae of both legs 5 days after the OVA injections. The lymph nodes were gently crushed in RPMI 10 and the resulting cell suspension filtered and used as lymph node cells for experiments. Expression of cytokines in the lymph node cells was confirmed by ELISA and RT-PCR.

Quantification of interferon- γ and interleukin-4 production by lymph node cells

Lymph node cells were adjusted to 1×10^6 cells/mL in RPMI 10. The cultures (0.2 mL/well) were incubated in 96-well culture plates (Nunc, Roskilde, Denmark) in the presence of Dynabeads Mouse T-Activator CD3/CD28 (Invitrogen Dynal AS, Oslo, Norway) at 37°C in a humidified atmosphere with 5% CO₂. The culture supernatants were collected after incubation for 48 hr, and the IFN- γ and IL-4 concentrations measured using ELISA kits for quantification of murine IFN- γ and IL-4, respectively (R & D Systems, Minneapolis, MN, USA).

Detection of interferon- γ and interleukin-4 mRNA expression in lymph node cells

In order to determine the degree of expression of IFN- γ and IL-4 mRNA, mRNA was extracted from lymph node cells using a Quick Prep Micro mRNA purification kit (Amersham Biosciences, Piscataway, NJ, USA). Then, cDNA was synthesized from 160 ng of the mRNA using a first-strand cDNA synthesis kit (Amersham Biosciences). PCR was performed using the following primers: β -actin (540 bp) 5' primer, 5'-GTGGGCCGCTCTAGGCACCAA-3' and 3' primer, 5'-CTCTTTGATGTCACGCACGATTTC-3'; IFN- γ (405 bp) 5' primer, 5'-GCTACACACTGCATCTTGGCTTTG-3' and 3' primer, 5'-CACTCGGATGAGCTCATTGAATGC-3'; IL-4 (400 bp) 5' primer, 5'-AGTTGTCATCCTGCTCTTCTTTCTC-3' and 3' primer, 5'-CGAGTAATCCATTTGCATGATGCTC-3'. Each PCR was performed using a GeneAmp PCR System 9700 (Perkin-Elmer, Norwalk, CT, USA) in 25 μ L of reaction mixture comprising 1.5 μ L cDNA (corresponding to 16 ng mRNA starting material), 200 μ M deoxynucleotide triphosphate mixture, 400 nM each PCR primer and 25 U/mL Ex *Taq* DNA polymerase (Takara, Shiga, Japan). The reaction conditions were as follows: one 4 min cycle at 94°C, 35 cycles comprising 45 s at 94°C, 45 s at 61°C and 2 min at 72°C, followed by one 7 min cycle at 72°C, after which the PCR products were separated on a 2% agarose gel containing ethidium bromide.

Quantification of interleukin-10 and -12 production by Langerhans cells

Langerhans cells were adjusted to 5×10^5 cells/mL in RPMI 10. The cultures (0.2 mL/well) were incubated in 96-well plates (Nunc) in the presence or absence of 10 μ g/mL PEG or 1 μ g/mL LPS at 37°C in a humidified atmosphere with 5% CO₂. Furthermore, in some experiments, LCs were incubated in the presence of

5 μ g/mL rat anti-mouse IL-10 neutralizing monoclonal antibody (clone JES5-16E3, IgG2a) (BioLegend, San Diego, CA, USA). Rat anti-keyhole limpet hemocyanin monoclonal antibody (clone B39-4, IgG2a) (BD Pharmingen, San Diego, CA, USA) was also used as an isotype-matched control antibody. The culture supernatants were collected after incubation for 48 hr and the IL-10, IL-12 p40 and IL-12 p70 concentrations measured using ELISA kits for quantification of each murine cytokine (R & D Systems).

Detection of T helper 2 adjuvant activity of peptidoglycan and influence of neutralization of interleukin-10 activity

T helper 2 adjuvant activity of PEG was detected according to the method of Whelan *et al.* (21) with modifications. LCs separated from the epidermis as described above were adjusted to 1×10^5 cells/mL in RPMI 10 and then stimulated with 10 μ g/mL PEG for 18 hr at 37°C in a humidified atmosphere with 5% CO₂. Furthermore, in some experiments, LCs were incubated in the presence of 5 μ g/mL rat anti-mouse IL-10 neutralizing monoclonal antibody (clone JES5-16E3, IgG2a) (BioLegend). Rat anti-keyhole limpet hemocyanin monoclonal antibody (clone B39-4, IgG2a) (BD Pharmingen) was also used as an isotype-matched control antibody. Next, Th cells were separated from DO 11.10 TCR Tg mouse spleen cells using an EasySep Negative Mouse CD4⁺ T Cell Enrichment Kit (StemCell Technologies, Vancouver, BC, Canada) and then treated with mouse anti-CD62L monoclonal antibody (clone lam1-116, IgG2a) (1 μ g per 1×10^6 cells; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in RPMI 10 for 1 hr on ice. The Th cells that had been reacted with anti-CD62L antibody were then purified using a CELlection Pan Mouse IgG Kit (Invitrogen Dynal AS), and used as naïve Th cells. Naïve Th cells (5×10^5 cells/mL) were cultured with the above-described PEG-stimulated LCs in the presence of 30 nM OVA peptide (323-ISQAVHAA-HAEINEAGR-339; obtained from Operon Biotechnologies, Tokyo, Japan) for 5 days at 37°C. The cells were then stimulated with 50 ng/mL PMA (Sigma) and 500 ng/mL ionomycin (Sigma) for 24 hr at 37°C. The cell supernatants were finally removed and tested for production of IFN- γ and IL-4 using ELISA kits (R&D Systems).

Statistical analysis

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

RESULTS

Effects of peptidoglycan on T helper 2 development

We purified LCs from BALB/c murine epidermis and pulsed them with OVA for 18 hr in the presence or absence of PEG or LPS. We injected the OVA-pulsed LCs into the hind footpads of BALB/c mice and gave the mice a booster injection of OVA into the hind footpads 2 days later. We harvested draining popliteal lymph nodes after a further 5 days, stimulated the T-lymphocytes among the lymph node cells thus obtained through cell surface CD3/CD28 molecules and determined IFN- γ and IL-4 concentrations in the culture supernatants by ELISA. As shown in Figure 1a, the PEG-stimulated LCs induced Th2-prone immune responses in a dose-dependent manner at 0.1–10 $\mu\text{g/mL}$, as represented by enhanced IL-4 production. However, the LPS-stimulated LCs most strongly induced Th1-prone immune responses at 1 $\mu\text{g/mL}$, as represented by enhanced IFN- γ production. We therefore performed subsequent experiments using 10 $\mu\text{g/mL}$ PEG and 1 $\mu\text{g/mL}$ LPS, after which we confirmed expressions of IFN- γ and IL-4 mRNA by RT-PCR. The data in Figure 1b indicate that LCs

stimulated with PEG induced a Th2-prone immune response, as shown by enhanced expression of IL-4 mRNA. However, LCs stimulated with LPS induced a Th1-prone immune response, as shown by enhanced expression of IFN- γ mRNA. These findings indicate that LCs stimulated with LPS and PEG induce development of Th1- and Th2-type cells, respectively.

Production of interleukin-12 from Langerhans cells upon stimulation with peptidoglycan and lipopolysaccharide

There is evidence that cytokines in the environment strongly influence maturation of Th cell precursors into biased Th1 or Th2 populations (22). In particular, IL-12 appears to be the dominant cytokine driving the differentiation of Th1 lymphocytes *in vitro* and *in vivo* (23, 24). We found that PEG and LPS stimulation of LCs induces significant amounts of IL-12 p40 and IL-12 p70, respectively (Fig. 2). However, PEG-stimulated LCs produced more IL-12 p40 than did LPS-stimulated LCs. Furthermore, PEG-stimulated LCs produced very much less IL-12 p70 than did LPS-stimulated LCs. Therefore, the ability to induce Th2-prone immune

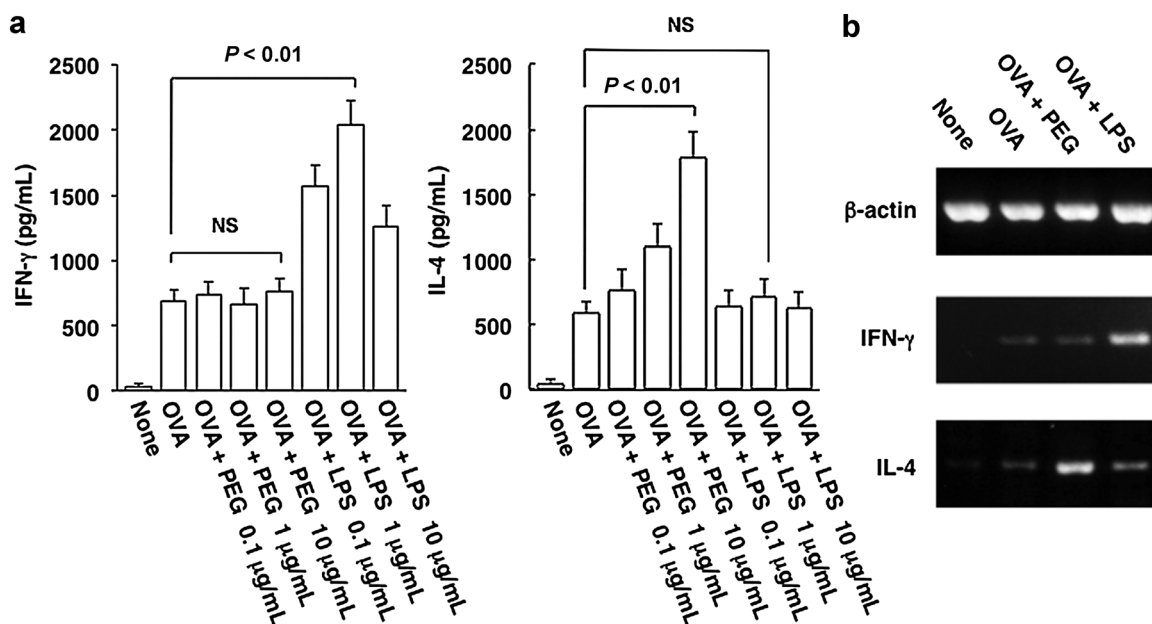


Fig. 1. Effect of peptidoglycan and lipopolysaccharide on T helper1/Th2 regulation in mice. (a) BALB/c mice that had been pre-stimulated with 0.1–10 $\mu\text{g/mL}$ PEG or LPS for 18 hr were primed with injections of OVA-pulsed LCs into the hind footpads and then given booster injections of OVA 2 days later via the hind footpads. Draining popliteal lymph node cells were harvested 5 days after OVA injection, stimulated through cell surface CD3/CD28 molecules for 48 hr, after which IFN- γ and IL-4 concentrations in the culture supernatants were determined by ELISA. The results are expressed as means $\pm$ SD ($n = 6$). (b) BALB/c mice that had been pre-stimulated with 10 $\mu\text{g/mL}$ PEG or 1 $\mu\text{g/mL}$ LPS for 18 hr were primed with injections of OVA-pulsed LCs into the hind footpads and then given booster injections of OVA 2 days later via the hind footpads. Draining popliteal lymph nodes were harvested 5 days after OVA injection, after which cytoplasmic mRNA was extracted from lymph node cells, reverse-transcribed, and amplified by PCR using β -actin, IFN- γ and IL-4 primer sets. The data shown are the representative results of five independent experiments.

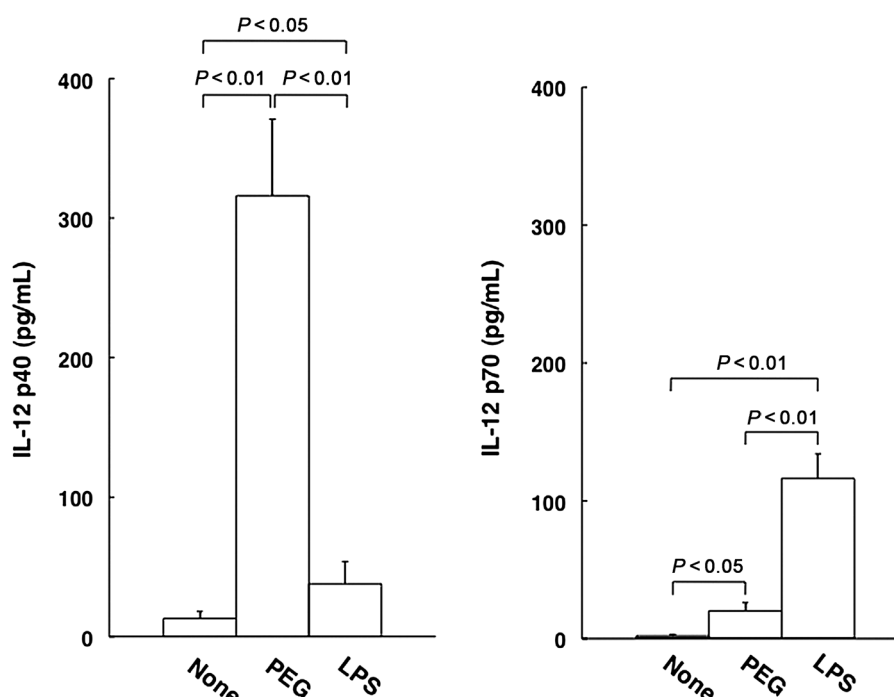


Fig. 2. Effects of peptidoglycan (PEG) and lipopolysaccharide (LPS) on interleukin(IL)-12 production by Langerhans cells. LCs (5×10^5 /mL) from BALB/c mouse epidermis were incubated with or without 10 μ g/mL PEG or 1 μ g/mL LPS for 48 hr. The supernatants were then assayed for IL-12 p40 and IL-12 p70 production using ELISA. The results are expressed as means $\pm$ SD ($n = 6$).

responses upon PEG-stimulation of LCs was mainly associated with little bioactive IL-12 and IL-12 p70 production and much IL-12 p40 production.

Production of interleukin-10 from Langerhans cells by stimulation with peptidoglycan and lipopolysaccharide

Some investigators have reported that IL-10 appears to be the dominant cytokine driving differentiation of Th2 lymphocytes *in vitro* and *in vivo* (25, 26). We found that PEG stimulation induces significant production of IL-10 by LCs (Fig. 3). However, we did not detect IL-10 production by LPS-stimulated LCs. Furthermore, neutralization of IL-10 activity with anti-IL-10 antibody production augmented production of both IL-12 p40 and IL-12 p70 by PEG-stimulated LCs (Fig. 4). Therefore, we surmise that the ability of PEG to induce Th2-prone immune responses in LCs is associated with down-regulation of IL-12 p70 production through IL-10 production.

Effect of neutralization of interleukin-10 activity on T helper 2 development

To examine whether Th2 development induced by PEG-stimulated LCs is associated with IL-10 production, we

studied the Th2 adjuvant activity of PEG and the influence of neutralization of IL-10 activity upon *in vitro*. As shown in Figure 5, PEG-stimulated LCs induced Th2-prone immune responses as represented by enhancement of IL-4 production, but not IFN- γ production, by activated CD4⁺ lymphocytes. Furthermore, neutralization of IL-10 activity with anti-IL-10 antibody inhibited this IL-4 production and conversely augmented IFN- γ production. These results suggest that Th2 development by PEG-stimulated LCs is associated with IL-10 production by LCs.

DISCUSSION

The number of Th2 cells is markedly increased in the peripheral blood and acute skin lesions of AD patients (1). Therefore, it is understood that development of Th2 cells plays an important causative role in AD. In this study, we investigated the capacity of mouse LCs to induce Th2-prone immune responses. The proposal that Th2-type immune responses play a key pathogenetic role in AD is supported by the presence of blood eosinophilia and high serum IgE concentrations in most AD patients (27). However, the immunoregulatory mechanism that induces Th2 development in AD is still unknown. In the present study, PEG stimulation induced

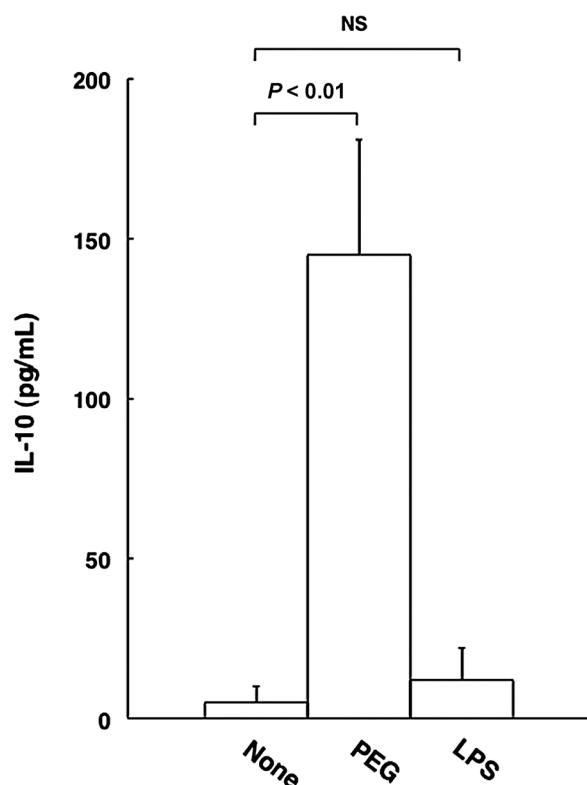


Fig. 3. Effects of peptidoglycan (PEG) and lipopolysaccharide (LPS) on interleukin (IL)-10 production by Langerhans cells. LCs ($5 \times 10^5/\text{mL}$) from BALB/c mouse epidermis were incubated with or without $10 \mu\text{g/mL}$ PEG or $1 \mu\text{g/mL}$ LPS for 48 hr. The supernatants were then assayed for IL-10 production using ELISA. The results are expressed as means $\pm$ SD ($n = 6$).

markedly less IL-12 p70 production by LCs than did LPS stimulation. This result is in accord with a previous report by Re *et al.* indicating that PEG stimulation of human DCs fails to induce IL-12 p70 but results in release of IL-12 p40 (28). Recent studies using mouse models have shown that activation of DCs by distinct TLR agonists appears to modulate the adaptive immune response. For example, activation of TLR2 by Pam3Cys or *Porphyromonas gingivalis* LPS induces prominent Th2-biased immune responses that are associated with failure of DCs to produce IL-12 p70 (29–31). Also, DCs activated with a superantigenic toxin, staphylococcal enterotoxin B, drive polarization of naïve allogeneic T cells into the Th2 subset *in vitro* (32). Furthermore, Th2 cell development is associated with DC activation through TLR2 signaling and absence of IL-12 p70 production. In contrast, *E. coli* LPS, which was used in our study and is known to be a Th1 adjuvant (29), induced Th1 immune responses associated with enhanced IL-12 p70 production. Therefore, it seems that down-regulation of IL-12 p70 production by antigen-presenting cells is important for

Th2 cell development. Our results suggest that production of IL-12 p40 by PEG-stimulated LCs together with absence of IL-12 p70 production may play critical roles in Th2 development. In fact, increased expression of IL-12 p40 protein has been observed in LCs in chronic AD skin lesions (33). Our previous study suggested that IL-12 p40 protein exists as a homodimer and works as an IL-12 p70 antagonist (9). Furthermore, our results demonstrate that the small amount of IL-12 p70 produced by PEG-stimulated LCs is associated with IL-10 production by these cells. IL-10 production by LCs probably induces down-regulation of IL-12 p70 production by these cells via an autocrine mechanism, resulting in inhibition of Th1 cell development and consequently of Th2 cell development. Therefore, PEG-induced Th2-prone immune responses would be inhibited by neutralization of IL-10 activity, resulting in restoration of IL-12 p70 production and consequently of Th1-prone immune responses. On the other hand, although production of IL-12 p40 by PEG-stimulated LCs is augmented by neutralization of IL-10 activity, subsequent Th1-prone immune responses are augmented rather than inhibited. Thus, further augmentation of IL-12 p40 production does not necessarily promote formation of the homodimer of p40 but instead promotes formation of the heterodimers of p35 and p40.

Since PEG is well known to be a TLR2 agonist (28), signaling through TLR2 on LCs would be associated with IL-10 production. In fact, we predicted the existence of TLR2 in LCs based on results of RT-PCR using a TLR2 primer set (data not shown). It has already been confirmed that the PEG used in this study is a specific stimulant of TLR2 (34). Because the density of *S. aureus* in skin lesions of AD patients exceeds 1×10^7 organisms/ cm^2 , the PEG concentration of $10 \mu\text{g/mL}$ we used for *in vitro* stimulation would realistically approximate the concentration *in vivo* (2). Therefore, sustained *S. aureus* colonization activates epidermal LCs to induce Th2-prone immune responses that are dependent on IL-10 production.

Previous studies have not sufficiently explained the role of *S. aureus* in the development of Th2 cells in patients with AD. The present results suggest that skin colonization with *S. aureus* plays a critical role in perpetuating skin tissue inflammation through development of Th2 cells induced by PEG, which is a common component of superantigenic exotoxin-producing and -non-producing *S. aureus* strains. Furthermore, superantigenic exotoxin-producing *S. aureus* strains would exert synergistic effects with PEG for induction of Th2 cell development. Because the superficial skin of most AD patients is colonized by *S. aureus* and has disrupted barriers because of reduced amounts of ceramide (35), PEG and superantigenic

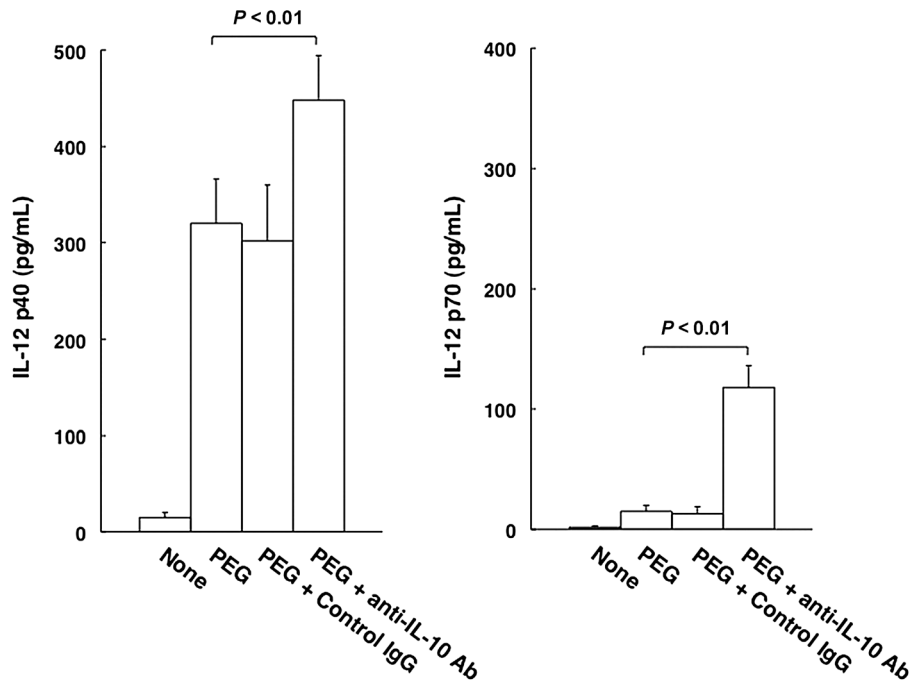


Fig. 4. Influences of interleukin (IL)-10 neutralization on IL-12 production by peptidoglycan (PEG)-stimulated Langerhans cells. LCs ($5 \times 10^5/\text{mL}$) from BALB/c mouse epidermis were incubated with $10 \mu\text{g/mL}$ PEG in the presence or absence of anti-IL-10 neutralizing antibody for 48 hr. The supernatants were then assayed for IL-12 p40 and IL-12 p70 production using ELISA. The results are expressed as means $\pm$ SD ($n = 6$).

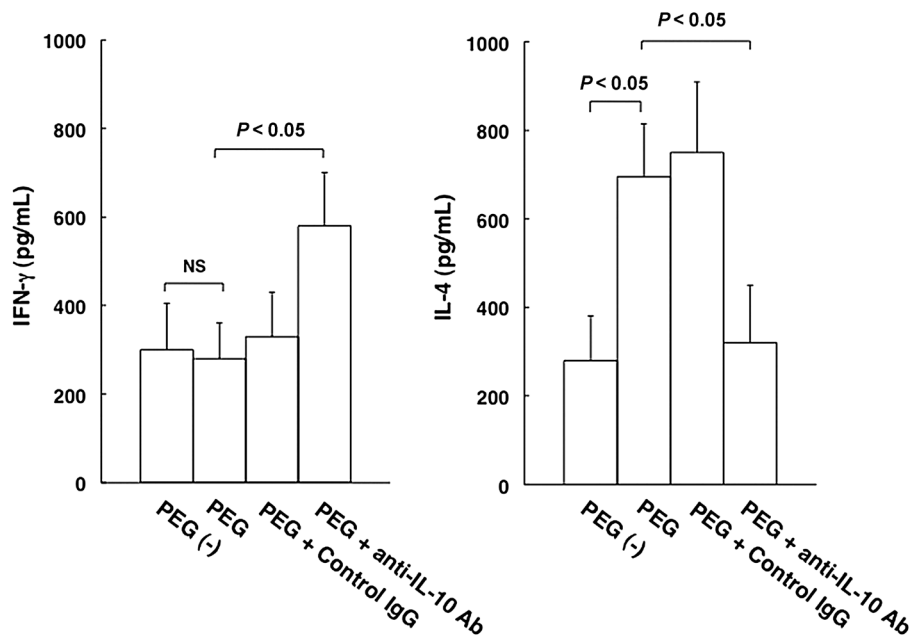


Fig. 5. Influences of interleukin (IL)-10 neutralization on T helper 2-prone immune responses induced by peptidoglycan (PEG)-stimulated Langerhans cells. LCs ($1 \times 10^5/\text{mL}$) from BALB/c mouse epidermis were incubated with $10 \mu\text{g/mL}$ PEG in the presence or absence of anti-IL-10 neutralizing antibody. After 18 hr, naïve Th cells from DO11.10 TCR Tg mouse ($5 \times 10^5/\text{mL}$) and 30 nM OVA peptide were added to the cultures and incubation continued for 5 days. The cells were then stimulated with PMA and ionomycin for 24 hr. The supernatants were assayed for IFN- γ and IL-4 production using ELISA. The results are expressed as means $\pm$ SD ($n = 6$).

exotoxin would be expected to penetrate continuously into the skin. Therefore, antimicrobial treatment in a subgroup of AD patients, irrespective of whether they show clinical signs of superinfection, could be a new therapeutic strategy for AD.

DISCLOSURE

The authors have no conflicts of interest.

REFERENCES

- Leung D.Y., Boguniewicz M., Howell M.D., Nomura I., Hamid Q.A. (2004) New insights into atopic dermatitis. *J Clin Invest* **113**: 651–7.
- Guzik T.J., Bzowska M., Kasproicz A., Czerniawska-Mysik G., Wójcik K., Szmyd D., Adamek-Guzik T., Pryjma J. (2005) Persistent skin colonization with *Staphylococcus aureus* in atopic dermatitis: relationship to clinical and immunological parameters. *Clin Exp Allergy* **35**: 448–55.
- Matsui K., Nishikawa A., Suto H., Tsuboi R., Ogawa H. (2000) Comparative study of *Staphylococcus aureus* isolated from lesional and non-lesional skin of atopic dermatitis. *Microbiol Immunol* **44**: 945–7.
- Sutcliffe I.C., Shaw N. (1991) Atypical lipoteichoic acids of gram-positive bacteria. *J Bacteriol* **173**: 7065–9.
- Fischer W. (1994) Lipoteichoic acids and lipoglycans. In: Ghuyssen J.M., Hackenbeck R., eds. *Bacterial Cell Wall*. Amsterdam: Elsevier Science Publishing, pp. 199–215.
- Matsui K., Motohashi R., Nishikawa A. (2000) Cell wall components of *Staphylococcus aureus* induce interleukin-5 production in patients with atopic dermatitis. *J Interferon Cytokine Res* **20**: 321–4.
- Matsui K., Nishikawa A. (2002) Lipoteichoic acid from *Staphylococcus aureus* induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen. *Clin Exp Allergy* **32**: 783–8.
- Matsui K., Nishikawa A. (2011) Percutaneous application of peptidoglycan from *Staphylococcus aureus* induces infiltration of CCR4⁺ cells into mouse skin. *J Invest Allergol Clin Immunol* **21**: 354–62.
- Matsui K., Nishikawa A. (2012) Peptidoglycan from *Staphylococcus aureus* induces Th2 immune response in mice. *J Invest Allergol Clin Immunol* **22**: 80–6.
- Merad M., Ginhoux F., Collin M. (2008) Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol* **8**: 935–47.
- Kang K., Kubin M., Cooper K.D., Lessin S.R., Trinchieri G., Rook A.H. (1996) IL-12 synthesis by human Langerhans cells. *J Immunol* **156**: 1402–7.
- Macatonia S.E., Hosken N.A., Litton M., Vieira P., Hsieh C., Culpepper J.A., Wysocka M., Trinchieri G., Murphy K.M., O'Garra A. (1995) Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4⁺ T cells. *J Immunol* **154**: 5071–9.
- D'Andrea A., Rengaraju M., Valiante N.M., Chehimi J., Kubin M., Aste M., Chan S.H., Kobayashi M., Young D., Nickbarg E., Chizzonite R., Wolf S.E., Trinchieri G. (1992) Production of natural killer cell stimulatory factor (interleukin-12) by peripheral blood mononuclear cells. *J Exp Med* **176**: 1387–98.
- Gubler U., Chua A.O., Schoenhaut D.S., Dwyer C.M., McComas W., Motyka R., Nabavi N., Wolitzky A.G., Quinn P.M., Familletti P.C., Gately M.K. (1991) Coexpression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation fraction. *Proc Natl Acad Sci USA* **88**: 4143–7.
- D'Andrea A., Ma X., Aste-Amezaga M., Paganin C., Trinchieri G. (1995) Stimulatory and inhibitory effects of interleukin (IL)-4 and IL-13 on the production of cytokines by human peripheral blood mononuclear cells: priming for IL-12 and tumor necrosis factor alpha production. *J Exp Med* **181**: 537–46.
- Gillessen S., Carvajal D., Ling P., Podlaski F.J., Stremlo D.L., Familletti P.C., Gubler U., Presky D.H., Stern A.S., Gately M.K. (1995) Mouse interleukin-12 (IL-12) p40 homodimer: a potent IL-12 antagonist. *Eur J Immunol* **25**: 200–6.
- Ling P., Gately M.K., Gubler U., Stern A.S., Lin P., Hollfelder K., Su C., Pan Y.C., Hakimi J. (1995) Human IL-12 p40 homodimer binds to the IL-12 receptor but does not mediate biologic activity. *J Immunol* **154**: 116–27.
- Heinzel F.P., Hujer A.M., Ahmed F.N., Rerko R.M. (1997) *In vivo* production and function of IL-12 p40 homodimers. *J Immunol* **158**: 4381–8.
- Tada Y., Asahina A., Nakamura K., Tomura M., Fujiwara H., Tamaki K. (2000) Granulocyte/macrophage colony-stimulating factor inhibits IL-12 production of mouse Langerhans cells. *J Immunol* **164**: 5113–9.
- Maldonado-López R., De Smedt T., Michel P., Godfroid J., Pajak B., Heirman C., Thielemans K., Leo O., Urbain J., Moser M. (1999) CD8α⁺ and CD8α[−] subclasses of dendritic cells direct the development of distinct T helper cells *in vivo*. *J Exp Med* **189**: 587–92.
- Whelan M., Harnett M.M., Houston K.M., Patel V., Harnett W., Rigley K.P. (2000) A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J Immunol* **164**: 6453–60.
- O'Garra A. (1998) Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* **8**: 275–83.
- Hsieh C.S., Macatonia S.E., Tripp C.S., Wolf S.F., O'Garra A., Murphy K.M. (1993) Development of Th1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* **260**: 547–9.
- O'Garra A., Murphy K.M. (2009) From IL-10 to IL-12: how pathogens and their products stimulate APCs to induce T(H)1 development. *Nat Immunol* **10**: 929–32.
- Iwasaki A., Kelsall B.L. (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* **190**: 229–39.
- Laouini D., Alenius H., Bryce P., Oettgen H., Tsitsikov E., Geha R.S. (2003) IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. *J Clin Invest* **112**: 1058–66.
- Grewe M., Bruijnzeel-koomen C.A.F.M., Schöpf E., Thepen T., Langeveld-Wildschut A.G., Ruzicka T., Krutmann J. (1998) A role of Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* **19**: 359–61.
- Re F., Strominger J.L. (2001) Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* **276**: 37692–9.
- Pulendran B., Kumar P., Cutler C.W., Mohamadzadeh M., Van Dyke T., Banchereau J. (2001) Lipopolysaccharides from distinct pathogens induce different classes of immune responses *in vivo*. *J Immunol* **167**: 5067–76.
- Redecke V., Hacker H., Datta S.K., Fermin A., Pitha P.M., Broide D.H., Raz E. (2004) Cutting edge: activation of Toll-like receptor

- 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* **172**: 2739–43.
31. Dillon S., Agrawal A., Van Dyke T., Landreth G., MaCauley L., Koh A., Maliszewski C., Akira S., Pulendran B.A. (2004) Toll-like receptor 2 ligand stimulates Th2 responses *in vivo*, via induction of extracellular signal-regulated kinase mitogen-activated protein kinase and c-Fos in dendritic cells. *J Immunol* **172**: 4733–43.
32. Mandron M., Ariès M.F., Brehm R.D., Tranter H.S., Acharya K. R., Charveron M., Davrinche C. (2006) Human dendritic cells conditioned with *Staphylococcus aureus* enterotoxin B promote Th2 cell polarization. *J Allergy Clin Immunol* **117**: 1141–7.
33. Yawalkar N., Karlen S., Egli F., Brand C.U., Graber H.U., Pichler W.J., Braathen L.R. (2000) Down-regulation of IL-12 by topical corticosteroids in chronic atopic dermatitis. *J Allergy Clin Immunol* **106**: 941–7.
34. Alexopoulou L., Holt A.C., Medzhitov R., Flavell R.A. (2001) Recognition of double-strand RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* **413**: 732–8.
35. Imokawa G., Abe A., Jin K., Higaki Y., Kawashima M., Hidano A. (1991) Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? *J Invest Dermatol* **96**: 523–6.