

Effects of Low-Level Laser Irradiation in a Mouse Model of Allergic Rhinitis

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Background and Objectives: To evaluate the anti-allergic effect of low-level laser irradiation (LLLI) at 650 nm in a mouse model of allergic rhinitis (AR), and to examine the underlying mechanisms.

Study Design/Materials and Methods: BALB/c mice were sensitized with ovalbumin (OVA) and alum and challenged intranasally with OVA. Straight- and diffusion-type LLLI were applied directly into the intranasal cavity of the mice once daily for 10 days (650 nm, 5 mW, 15 min/day) and multiple allergic parameters were evaluated.

Results: LLLI reduced allergic symptoms, such as rubbing and sneezing, and suppressed the serum total immunoglobulin E (IgE), OVA-specific IgE, and OVA-specific IgG1 levels. Diffusion-type LLLI significantly reduced eosinophil infiltration of nasal mucosa and lymph nodes (LNs). LLLI reduced the expression of interleukin-4 (IL-4) and IL-17 in cervical LN and splenocyte culture supernatant, as well as their messenger RNA levels in nasal mucosa. However, the expression of interferon- γ (IFN- γ) and IL-6 was unaffected by LLLI. The levels of reactive oxygen species (ROS) and nitric oxide (NO) in LN cells and the nasal mucosa, which were increased in the AR group, were reduced by LLLI, suggesting involvement of ROS and NO within their mechanism.

Conclusions: LLLI exerted an antiallergic effect by decreasing local and systemic IL-4, IL-17, and IgE levels, as well as eosinophilic infiltration into the nasal mucosa, in a mouse model of AR by modulating ROS and NO levels. Diffusion-type LLLI exhibited greater efficacy against AR than straight-type LLLI. *Lasers Surg. Med.* © 2019 Wiley Periodicals, Inc.

Key words: allergic rhinitis; low level laser irradiation; diffusion; straight; reactive oxygen species; nitric oxide

INTRODUCTION

Allergic rhinitis (AR) is one of the most common disease characterized by sneezing, itching, rhinorrhea, and nasal obstruction. It is mediated by immunoglobulin E (IgE) and T-helper type 2 (Th2)-mediated allergic inflammation [1]. The prevalence of AR is increasing worldwide and its economic burden has increased these days [2]. Although

several treatment modalities have been tried to control allergic rhinitis including avoidance, pharmacotherapy, and immunotherapy, some patients with AR could not be treated properly. Therefore, several efforts for new therapeutic modalities are investigated.

Low-level laser irradiation (LLLI) is one of the adjunct treatment modalities and has been used clinically in several clinical fields to aid wound healing [3], to relieve pain caused by musculoskeletal diseases [4,5], and to stimulate acupuncture points [6]. Moreover, the anti-inflammatory and immunosuppressive effects of LLLI have been reported not only in *in vitro* studies but also in animal model of allergic inflammation [7–9]. And there have been some clinical trials that have used LLLI in patients with AR.

Although LLLI has been used clinically for the treatment of AR, its effect and mechanism are not clear. Moreover, few studies have evaluated the antiallergic effects of LLLI. Therefore, the effect of laser irradiation on the nasal mucosa of ovalbumin (OVA)-induced allergic rhinitis in mouse model was investigated using both straight- and diffusion-type lasers of 650 nm wavelength in this study. In addition, we also tried to evaluate the mechanism underlying the antiallergic effect of LLLI.

MATERIALS AND METHODS

Laser device and application

The low-power 650 nm infrared laser device (Mirint Co., Ltd., Seongnam, Korea) had a poly-lactic-co-glycolic acid fiber of 0.5 mm diameter and an output of 5 mW. Laser irradiation

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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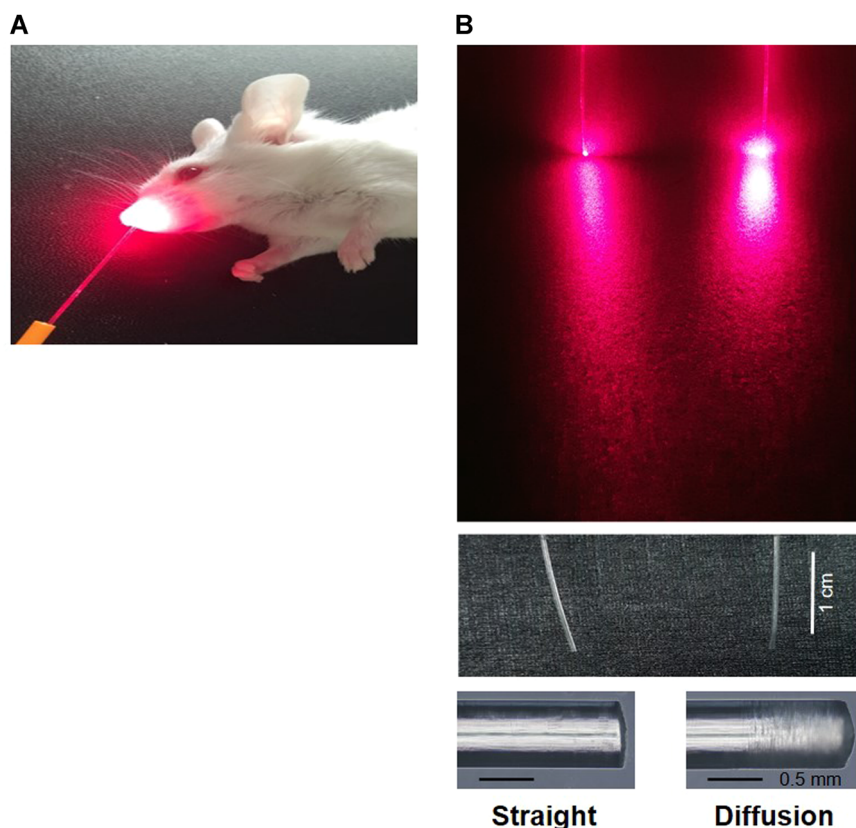


Fig. 1. Laser device and irradiation. (A) The mucosa of each nostril of an anesthetized mouse was irradiated in turns with the laser for 15 minutes. (B) Photographs of the nasal mucosa before and after irradiation with the straight- and diffusion-type laser and of the tips of the laser fibers. [Color figure can be viewed at wileyonlinelibrary.com]

with straight- and diffusion-type fibers was applied to the nasal cavity of the mice under anesthesia (Fig. 1A). Photographs of the tips of the diffusion- and straight-type laser devices and their range of irradiation are shown (Fig. 1B), and the laser irradiation parameters are listed in Table 1.

Mouse Model of AR and Treatments

Twenty female BALB/c mice (4 weeks of age, 18–20 g; Orient Bio Inc.; Seongnam, Republic of Korea) were divided into four groups ($n = 5$ each): negative control group, positive control group (AR induced by OVA + Alum), straight-type LLLI group, and diffusion-type LLLI group (Fig. 2A). The experiments were performed in triplicate. The AR model was induced using a protocol described previously with

minor modifications [10]. In brief, mice were sensitized by intraperitoneal injection of 25 μ g of OVA (grade V; Sigma-Aldrich, St. Louis, MO) and 1 mg of aluminum hydroxide gel on days 0, 7, and 14. Next, the mice were challenged intranasally with 100 μ g of OVA daily from days 21 to 25. After anesthesia, the optical fiber was inserted into the nasal passage. The mice in the laser-treatment groups were irradiated with a straight- or diffusion-type laser (4.5 J/day for 10 days), 3 hours before intranasal OVA challenge for 20 minutes. In case of mice in negative and positive group, optical fiber was inserted for 15 minutes without laser irradiation. The Committee on the Use and Care of Animals of our institution approved the animal experiments and we complied with the governmental and international guidelines on animal experimentation (DKU-2017-009).

Symptom Score and Tissue Preparation

After the final OVA challenge, a blinded observer recorded the frequencies of sneezing and nasal rubbing for 15 minutes. The mice were euthanized 24 hours after the last OVA challenge. Half of the head was removed *en bloc* and fixed in 4% paraformaldehyde. The nasal mucosa was removed from the nasal cavity of the other half of the head using a small curette, immediately immersed in TRIzol reagent (Invitrogen, Carlsbad, CA), and stored at -70°C until use for

TABLE 1. Laser Irradiation Parameters

Parameters	Values
Power output	5 mW
Wavelength	650 nm
Mode of action	Continuous
Irradiation time	15 min/day
Fiber diameter	0.5 mm

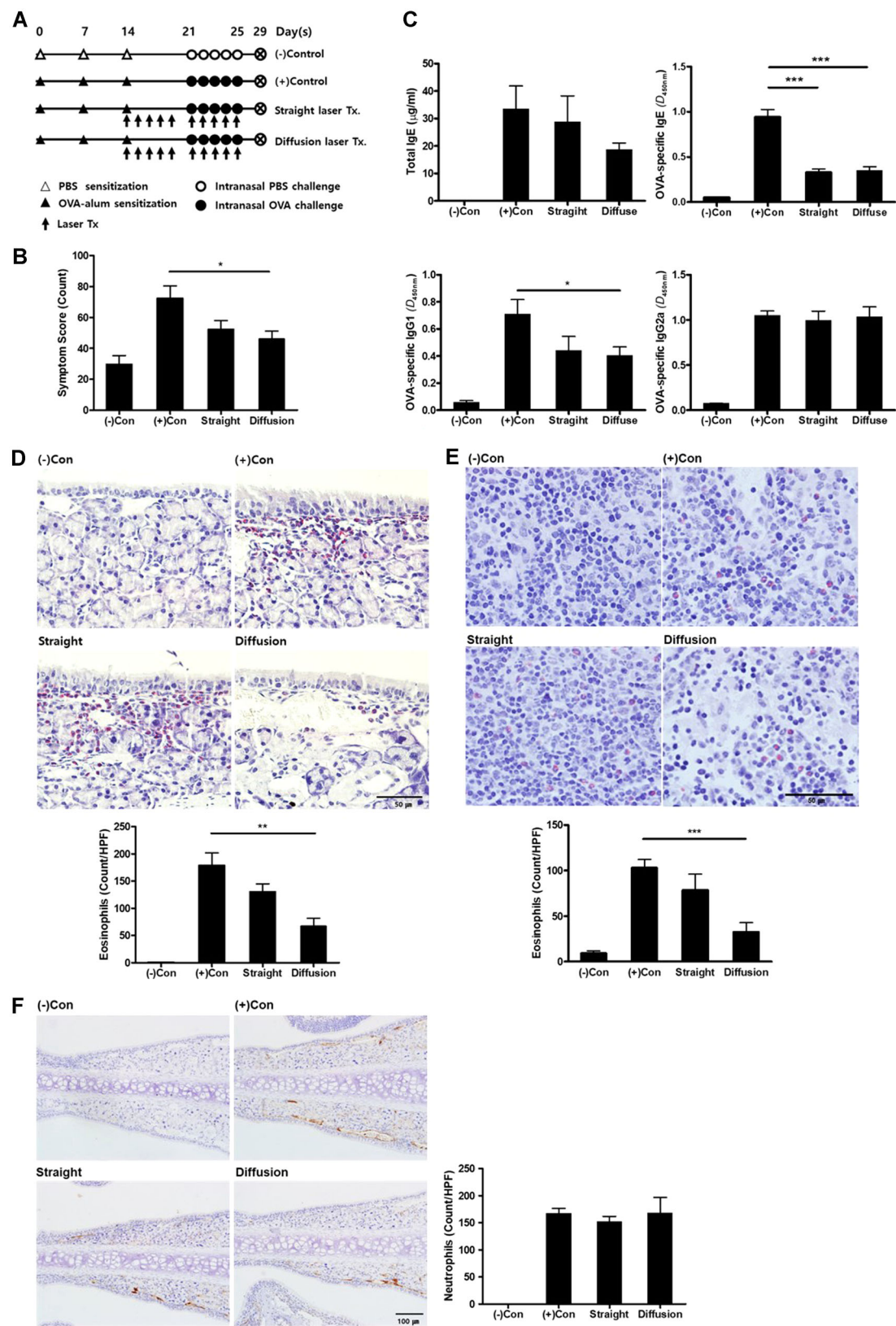


FIGURE 2 Continued.

reverse-transcription-quantitative reverse-transcription polymerase chain reaction (RT-qPCR). The cervical lymph nodes (LNs) were isolated and fixed in 4% paraformaldehyde for histological evaluation.

Eosinophils in the Nasal Septal Mucosa and Cervical LNs

For evaluation of nasal histology, nasal tissues were decalcified, embedded in paraffin, and sectioned coronally (4- μ m thick), approximately 5 mm from the nasal vestibule. The sections were stained with Sirius red and the number of eosinophils on both sides of the septal mucosa was counted. The number of eosinophils in the submucosal area of the whole nasal septum was counted under a light microscope ($\times 400$ magnification). Fixed cervical LNs were embedded in paraffin, sectioned, and stained with Sirius red. The number of eosinophils in LNs was counted in 4–5 separate high-power fields and the mean values were calculated.

Immunohistochemistry

The nasal mucosa was immunostained using an antineutrophil antibody (NIMP-R14; Abcam, Cambridge, MA) and cervical LN sections were immunostained with anti-IL-4, -IL-17, and -IFN γ antibodies using Avidin-Biotinylated-Horseradish Peroxidase (HRP) kits (Vector Laboratories, Burlingame, CA). After deparaffinization in xylene, the sections were rehydrated through an ethanol gradient, washed in phosphate-buffered saline (PBS), blocked with 1% normal goat serum, and treated with the appropriate primary antibody (1:50 dilution) overnight at 4°C in a humidified chamber. After a further wash in PBS, the sections were incubated for 60 minutes at room temperature with the secondary biotin-conjugated goat anti-rat IgG (1:100 dilution). The sections were then incubated with avidin-biotinylated-HRP complex for 30 minutes at room temperature, rinsed in PBS, and developed with 0.027% 3,3'-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) and 0.003% hydrogen peroxide. Finally, the sections were counterstained with hematoxylin (Sigma-Aldrich) and imaged under a bright-field microscope at $\times 400$ magnification.

Assay of Cytokine Levels in the Nasal Mucosa

Total RNA was prepared from nasal mucosa using TRIzol reagent (Invitrogen). Complementary DNA (cDNA) was synthesized from 1 μ g of RNA using the iScript cDNA

Synthesis Kit (Bio-Rad, Hercules, CA). For analysis of IL-4 (Mm00445258_g1), IL-5 (Mm01290072_g1), IL-6 (Mm00446190_m1), IL-10 (Mm00439616_m1), IL-17 (Mm00439618_m1), IFN- γ (Mm99999071_m1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Mm03302249_g1), predeveloped assay reagent kits were used (Applied Biosystems, Foster City, CA). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed on the ABI 7500 Real-Time PCR System (Applied Biosystems). Mean transcript levels were normalized to that of GAPDH.

Measurement of Cytokine Profiles in Spleen

Single splenocyte suspensions were plated in 24-well cell-culture plates at a final concentration of 3×10^6 /ml in RPMI-1640 medium (Gibco, Grand Island, NY). The cells were incubated in a CO $_2$ atmosphere at 37°C and stimulated with OVA (1 mg/ml) for 72 hours. The culture supernatant was collected and stored at -70°C until required. Cytokine levels in the culture supernatant were assayed using a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) Kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. After measuring the optical density (OD) at 450 nm, the concentrations of IL-4, IL-5, IL-6, IL-10, IL-17, and IFN- γ were determined by interpolation from a standard curve and are expressed as ng/ml.

Serum total IgE, OVA-specific IgE, IgG1, and IgG2a

The serum levels of total and OVA-specific IgE were measured by solid-phase ELISA. Serum samples collected at the time of euthanasia were serially diluted and added to 96-well plates coated with purified anti-mouse IgE monoclonal antibody (clone R35-72; BD Pharmingen, San Jose, CA). A purified mouse IgE isotype (27-74; BD Pharmingen) was used as the standard for total IgE. Nonspecific antigen-antibody reactions were blocked with 3% bovine serum albumin. To detect total IgE, an HRP-conjugated anti-mouse IgE (23G3; Southern Biotechnology, Birmingham, AL) was added. To detect OVA-specific IgE, biotin-labeled OVA was added, followed by an HRP-labeled anti-biotin antibody (Vector Laboratories). The reactions were developed using 3,3',5,5'-tetramethylbenzidine (Moss, Inc., Belfast, ME) and terminated by adding 2 N H $_2$ SO $_4$. The OD at 450 nm was recorded using a luminometer (iEMS Reader;

Fig. 2. Experimental protocol and effects of low-level laser irradiation (LLI) on the allergic symptom score, serum levels of immunoglobulins (Igs), and eosinophil and neutrophil infiltration in the nasal mucosa and cervical lymph nodes (LNs). (A) BALB/c mice were sensitized with ovalbumin (OVA) and 1 mg of Alum on days 0, 7, and 14 (general sensitization). All groups other than the negative control group underwent intranasal administration of OVA from days 21–25. The mice in the laser treatment group were irradiated with the straight- or diffusion-type laser (4.5 J/15 min/day for 10 days), 3 hours before intranasal OVA challenge. (B) Frequencies of sneezing and nasal rubbing after the final OVA challenge. (C) Serum levels of total IgE and OVA-specific Ig (E, G1, and G2a) by enzyme-linked immunosorbent assay (ELISA). Representative photographs of Sirius-red-stained sections, and numbers of eosinophils in nasal tissue (D) and cervical LNs (E) ($\times 400$ magnification). (F) Representative images of neutrophil infiltration and the number of neutrophils in nasal tissue by IHC ($\times 200$ magnification). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, Mann-Whitney test. [Color figure can be viewed at wileyonlinelibrary.com]

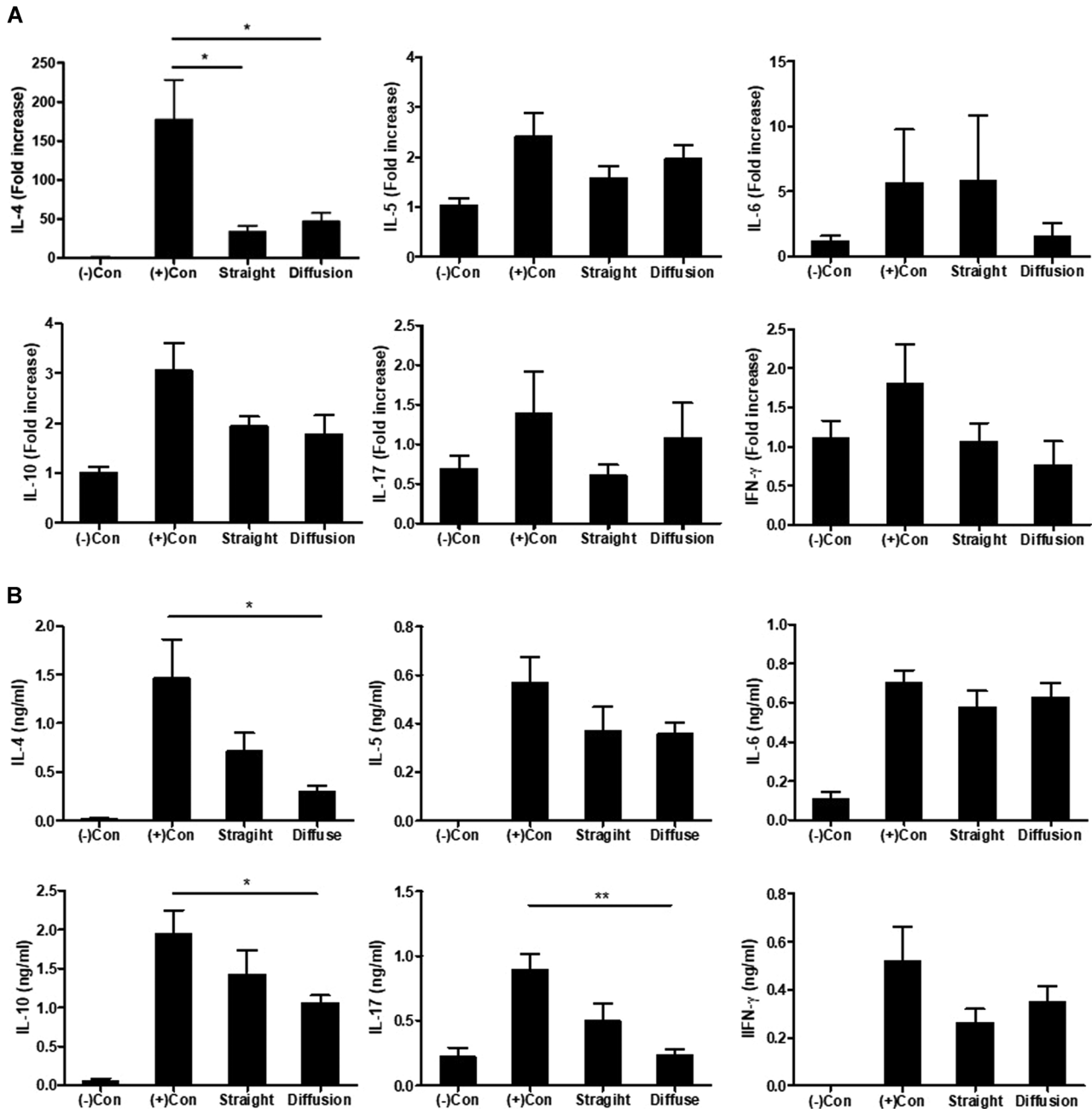


FIGURE 3 Effects of low-level laser irradiation (LLLI) on the protein and messenger RNA (mRNA) levels of proinflammatory cytokines in splenocyte culture supernatant, nasal mucosa, and cervical LNs. **(A)** interleukin-4 (IL-4), IL-5, IL-6, IL-10, IL-17, and interferony (IFN- γ) levels in the culture supernatant of spleen cells was determined by enzyme-linked immunosorbent assay (ELISA). The spleen cells were restimulated with ovalbumin (OVA). **(B)** Relative mRNA levels of cytokines in the nasal mucosa. Representative images of IL-4 **(C)**, IL-17A **(D)**, and IFN- γ **(E)** expression and staining intensity in cervical lymph nodes (LNs) by immunohistochemistry (IHC) ($\times 40$, low magnification; $\times 400$, high magnification). * $P < 0.05$ and ** $P < 0.01$ by Mann-Whitney test. [Color figure can be viewed at wileyonlinelibrary.com]

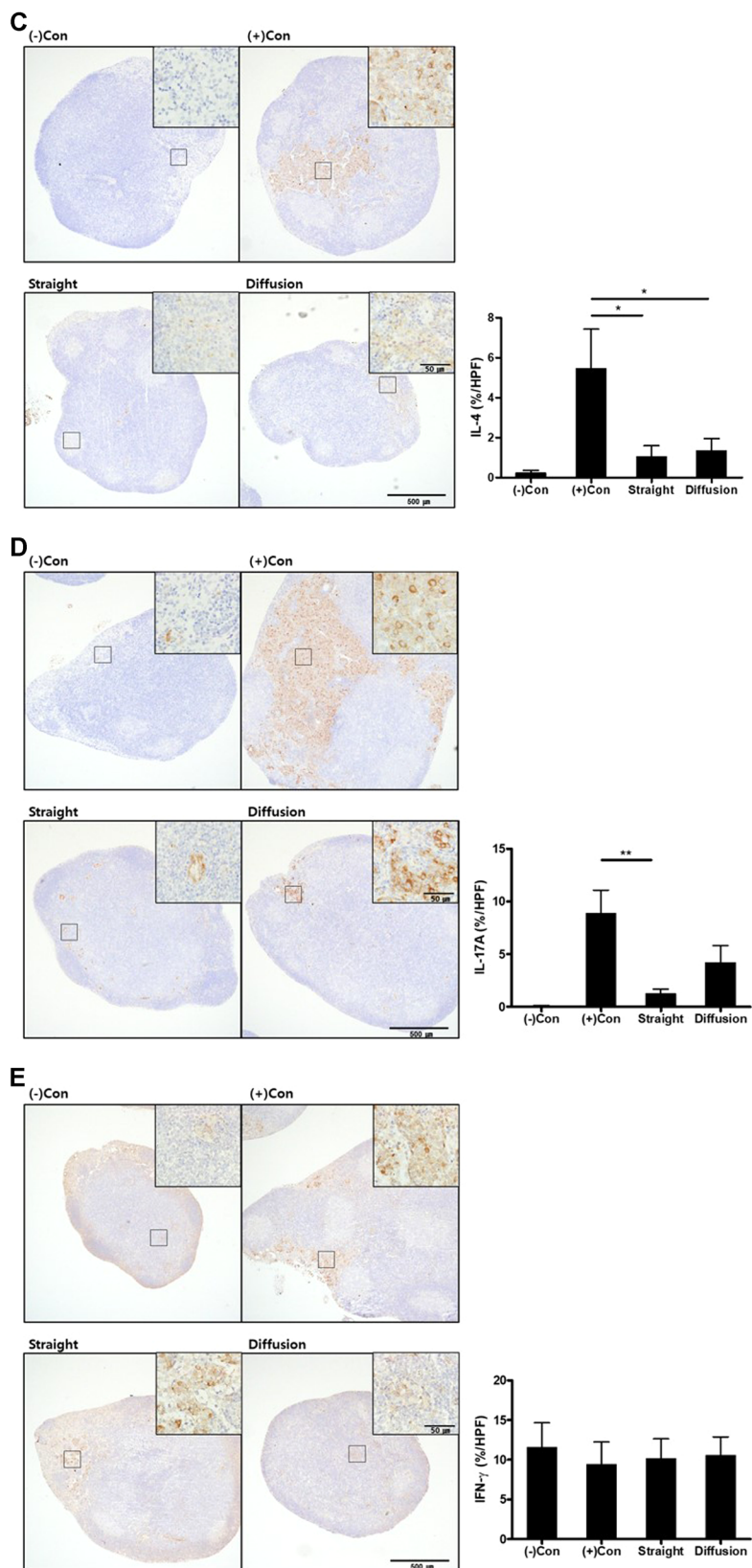


FIGURE 3 Continued

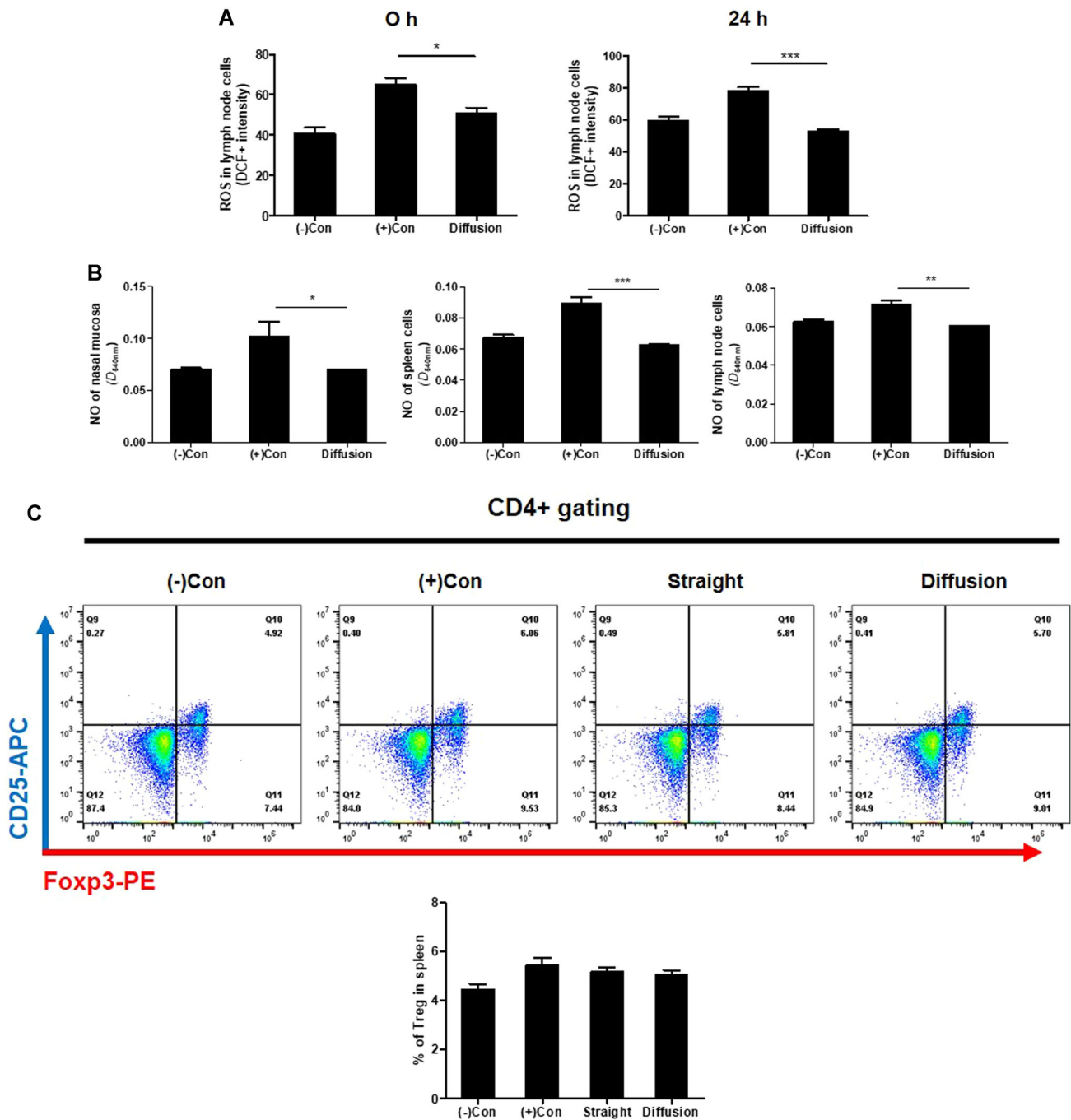


Fig. 4. Reactive oxygen species (ROS) levels in cervical lymph node (LN) cells, nitric oxide (NO) levels in the nasal mucosa, spleen, and cervical LN cells, and number of regulatory T cells (Tregs) among spleen cells. (A) Intracellular ROS levels in cervical LN cells cultured with ovalbumin (OVA) for 0 or 24 hours by flow cytometry. (B) NO levels in cell culture medium and nasal mucosa extract. LN and spleen cell culture medium was collected following incubation with OVA for 24 hours. (C) Frequency of Tregs ($CD4^+CD25^+Foxp3^+$) among spleen cells, and of $CD25^+$ and $Foxp3^+$ cells among $CD4^+$ T cells, by flow cytometry. [Color figure can be viewed at wileyonlinelibrary.com]

Labsystems, Helsinki, Finland). The endpoint titers of OVA-specific IgE are expressed as the reciprocal $\log_2$ of the last dilution of a sample that resulted in an OD value 0.1 higher than the background.

To assay OVA-specific IgG1 and IgG2a levels, 96-well immunoplates (Nunc) were coated with 100 $\mu\text{g}/\text{ml}$ OVA in coating buffer (carbonate-bicarbonate) overnight at 4°C. To detect OVA-specific IgG1 and IgG2a, serially diluted serum

samples were incubated with biotinylated rat anti-mouse IgG1 (BD Pharmingen) and IgG2a (BD Pharmingen), respectively, and streptavidin-HRP was added. SureBlue TMB Microwell Substrate (KPL, Gaithersburg, MD) was added for 30 minutes to detect peroxidase and promote color development. Finally, the reaction was stopped by adding 1 M HCl, and the OD was measured using a microplate reader (Tecan, Mannedorf, Switzerland).

Flow Cytometry

Spleen cells were stained with anti-CD4-FITC and anti-CD25-APC antibodies and a Foxp3/transcription factor staining buffer set was used to stain Foxp3. Surface-stained cells were treated with fixing and permeabilization buffer, followed by an anti-Foxp3-PE antibody. The frequency of CD4⁺/CD25⁺/Foxp3⁺ regulatory T cells (Tregs) in splenocytes was determined using an Accuri C6 flow cytometer (BD Bioscience, San Jose, CA). The antibodies and buffers used for flow cytometry were purchased from eBioscience (San Diego, CA).

To assay ROS levels, spleen cells were cultured with OVA for 24 hours and incubated in PBS with 10 μ M DCF-DA (Sigma-Aldrich) for 30 minutes. After washing in PBS, the percentage of DCF⁺ cells was analyzed by flow cytometry to determine the ROS level. Flow cytometry data were analyzed using FlowJo software (Treestar, San Carlos, CA).

Assay of Nitric Oxide Levels

The Griess reagent was used to measure NO levels in cell culture medium and nasal mucosa extract [11,12]. Culture medium of LN and spleen cells was collected following incubation with OVA for 24 hours. Nasal mucosa was centrifuged and mixed with PBS to prepare the nasal mucosa extract. Equal volumes of medium or extract and modified Griess reagent (Sigma-Aldrich) were mixed and after 15 minutes the OD at 540 nm was measured using a spectrophotometer.

Statistical analysis

Data are means $\pm$ standard deviations. The statistical significance of differences among groups was evaluated by Mann-Whitney *U* test. A value of *P* < 0.05 was considered as significant.

RESULTS

Allergic Symptoms and Serum Immunoglobulin Levels

The allergic symptom score was significantly higher in the positive control group compared with the negative control group. It was decreased by LLLI using either type of laser; the decrease was statistically significant for the diffusion-type laser (**P* < 0.05, Fig. 2B). Moreover, LLLI significantly decreased the level of OVA-specific IgE (Fig. 2C). OVA-specific IgG1 was decreased by LLLI using either type of laser and it was statistically significant for the diffusion-type laser (Fig. 2C). OVA-specific IgG2a was not changed by LLLI. Total IgE level showed a tendency to decrease in both LLLI groups without statistical

significance. LLLI decreased the allergic symptoms and IgE levels in mice with AR, and the diffusion-type laser showed greater efficacy than the straight-type laser.

Eosinophil Infiltration in the Nasal Mucosa and Cervical LNs

Eosinophil infiltration in the nasal mucosa and cervical LNs was significantly increased in the positive control group compared with the negative control group. The number of eosinophils in the nasal mucosa was significantly reduced by LLLI using the diffusion-type laser (Fig. 2D). Eosinophil infiltration of cervical LNs showed similar results with those of the nasal mucosa (Fig. 2E). However, neutrophil infiltration in the nasal mucosa was not altered by LLLI using either type of laser (Fig. 2F). Therefore, LLLI using the diffusion-type laser significantly decreased eosinophil infiltration in the nasal mucosa and cervical LNs.

Systemic and Local Cytokine Levels

The mRNA level of the Th2 cytokine, IL-4, in the nasal mucosa was decreased significantly by LLLI using either type of laser, whereas that of IL-5 decreased nonsignificantly. The mRNA levels of the Th1 and Th17 cytokines, IFN- γ and IL-17, were not significantly influenced by LLLI. The mRNA levels of IL-6 and IL-10 were also not significantly altered by LLLI (Fig. 3A).

The IL-4, IL-17, and IL-10 levels in splenocyte culture supernatant were significantly reduced by LLLI using the diffusion-type laser. However, LLLI using either type of laser did not significantly influence the IL-5, IL-6, and IFN- γ levels in splenocyte culture supernatant (Fig. 3B).

The IL-4 level in cervical LNs was significantly reduced by LLLI (Fig. 3C). The increased IL-17 level in the positive control group was decreased to a greater degree by LLLI using the straight-type compared with the diffusion-type laser (Fig. 3D). The IFN- γ level did not differ among the groups (Fig. 3E). Therefore, LLLI reduces the systemic and local levels of IL-4 and IL-17.

Changes in ROS and NO

Flow cytometry of cervical LN cells cultured for 0 or 24 hours showed that the increased ROS level in AR was decreased by LLLI (Fig. 4A). The NO levels in nasal mucosa extract and the culture supernatants of cervical LN and spleen cells were decreased by LLLI (Fig. 4B). However, LLLI did not significantly influence the CD25⁺Foxp3⁺CD4⁺ Treg frequency among the spleen cells (Fig. 4C). Therefore, LLLI ameliorates allergic inflammation by modulating the levels of ROS and NO but not the number of Tregs.

DISCUSSION

Although the mechanism of action of LLLI is unclear and its clinical application is subject to several limitations, it is reportedly efficacious against a variety of conditions. LLLI is a useful alternative treatment for pain, inflammation, and wounds; however, scientific evidence is still lacking.

Photobiomodulation [13] influences mitochondrial cytochrome *c* oxidase activity [14], NO [15], and ATP [16] levels. LLLI modulates the intracellular concentrations of calcium ions and ROS, as well as the expression of redox-sensitive transcription factors such as nuclear factor- κ B (NF- κ B) [17–19]. Excess ROS is implicated in the pathogenesis of airway inflammation [17,20–22]. However, the association between ROS and AR is unclear. Tregs may prevent allergic disorders by maintaining immune homeostasis [23,24]

Although it is used not only clinically but also as a self-treatment medical device for diseases including AR, it is important to identify the mechanism of action. In this study, we evaluated the effect of LLLI using a straight- or diffusion-type 650 nm laser in a mouse model of AR and examined the underlying mechanism. We first assessed and compared the effects of straight- and diffusion-type lasers in the AR mouse model, and confirmed the possibility of mechanisms through ROS and NO pathways. In addition, we found that the effects of mouse AR treatment may be different depending on the type of laser irradiation.

The symptom scores and serum Ig levels of AR mice were decreased by LLLI using either type of laser (Fig. 2B and C). Moreover, the symptom scores, and the serum OVA-specific IgG1 level, were significantly reduced by LLLI using the diffusion-type laser. Similar to our results, one study reported that the patients with AR received intranasal illumination at 660 nm for 4.4 minutes, three times a day for 14 days (total dose 6 J/day), and had symptom improvement, and claimed that the marked alleviation of clinical symptoms due to illumination could effectively treat AR [25]. In previous studies using the mouse AR model, the level of total IgE and IL-4 in serum was significantly reduced by LLLI (658 nm, 30 mW, 15×10^{-2} W/cm², 320 s/day, 7 days) [26].

Similar to the results of reducing the eosinophils as well as inflammatory cytokines (IL-4, IL-5, and IL-13) in the BAL fluid by applying LLLI (660 nm, 8 J/cm²/day, for 21 days) to the rat asthma model [27], the increased eosinophil infiltration in the nasal mucosa in AR was significantly reduced by LLLI using the diffusion-type laser in our experiments (Fig. 2D). Moreover, the levels of inflammatory cytokines in the culture supernatant of spleen cells were reduced by LLLI (Fig. 3B). Especially, the levels of the Th2-related cytokine IL-4, and those of the Th17-related cytokines IL-17 and IL-10, were significantly decreased by LLLI using the diffusion-type laser. In addition, the mRNA levels of most of the cytokines in the nasal mucosa were reduced by LLLI, and the reduction in the IL-4 mRNA level was statistically significant (Fig. 3A), suggesting that LLLI has inhibitory effects on inflammatory cytokines production in the AR mouse model.

The cervical LNs, which are those closest to the nose, reflect the inflammatory response in AR [28,29]. We evaluated the effect of LLLI by evaluating the levels of eosinophil infiltration and inflammatory cytokines in cervical LN. As a result, LLLI with the diffusion-type

laser significantly decreased eosinophil infiltration of LN tissues (Fig. 2E). Also, LLLI using the straight- and diffusion-type lasers reduced the IL-4 level, while the IL-17 level was significantly decreased by LLLI only by the straight-type laser in the LN (Fig. 3C and D).

Our results showed that there was a discrepancy between the nasal mucosa neutrophil infiltration (Fig. 2F) and cervical LN IL-17 expression (Fig. 3D). Usually IL-17 were closely related with neutrophil infiltration. However, although LN IL-17 was diminished with laser treatment, nasal mucosal neutrophil infiltration was not. It is suggested that the inflammatory response of each tissue can be reflected differently because each tissue respond differently to the laser treatment. And the timing of sacrifice could have influenced this discrepancy.

LLLI stimulates healing, relieves pain, and ameliorates inflammation by modulating ROS and NO levels [15,19,30,31]. Therefore, we evaluated the ROS and NO levels in the nasal mucosa, cervical LNs, and spleen. Eosinophils from the nasal cavity of patients with AR showed elevated production of ROS [32] and an excess of ROS serve an important role in the pathogenesis of airway inflammation [33–36]. Also, nasal NO levels in patients with AR are significantly higher than those of healthy control subjects [37], and ozone produced from NO by the action of sunlight reportedly exacerbates the symptoms of AR [38]. Several studies have shown that LLLI regulates allergic lung inflammation via signal transducer and activator of transduction 6 in mouse allergic asthma models [39] and starts the modulation of physiological processes incrementing the production of ATP, which stimulates the microcirculation, increases collagen fiber deposition, promotes higher fibroblast cell proliferation in the site of the lesion, and reduces the number of inflammatory cells [40–44].

In this study, LLLI significantly decreased the ROS level in OVA-stimulated LN cells from mice with AR (Fig. 4A). Also, LLLI reversed the increased NO levels in the nasal mucosa extract and culture supernatant of LNs and splenocytes from the mice with AR (Fig. 4B).

Tregs play a fundamental role in maintaining the balance of the immune system to prevent allergic disorders [23,24], so we investigated whether LLLI could affect the Treg population. However, the Treg (CD4+ Foxp3+ CD25+) populations in spleen did not differ among the groups in this our experimental conditions (Fig. 4C).

In our experiment, the diffusion-type lasers reduced the allergic inflammatory response more than the straight-type lasers. It is expected that the irradiated mucosal area by diffusion-type lasers can be wider than that by the straight-type lasers, thus resulting in increased antiallergic effects by diffusion-type lasers.

In conclusion, LLLI reduced the allergic symptoms; local and systemic IL-4 and IL-17; serum Ig levels; and eosinophil infiltration into the nasal mucosa by modulating ROS and NO levels in mice with AR. Also, the diffusion-type laser showed greater efficacy than the straight-type. These results support the clinical efficacy of LLLI. The mechanism of action of LLLI in various

diseases, including AR, needs to be clarified in further studies.

Finally, the present study demonstrated for the first time that diffusion-type laser treatment relieves nasal inflammation in a mouse model of AR.

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