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Changes in immune responses to antigen applied to tape-stripped skin with CpG-oligodeoxynucleotide in mice

Joe Inoue, Satoshi Yotsumoto, Takatoshi Sakamoto,
Seishi Tsuchiya, Yukihiko Aramaki *

School of Pharmacy, Tokyo University of Pharmacy and Life Science 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

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Abstract

CpG-oligodeoxynucleotide (CpG-ODN) plays a critical role in immunity via the augmentation of Th1 and suppression of Th2 responses. We examined here the effect of CpG-ODN on the immune response to an antigen applied to tape-stripped mouse skin by evaluating the production of cytokines and Ig isotypes. Confocal laser scanning microscopy revealed that the model antigen, OVA, and CpG-ODN easily penetrated the tape-stripped skin. Co-administration of CpG-ODN and OVA to the disrupted skin elicited an antigen-specific Th1-predominant immune response and enhanced the production of Th1-type cytokines, IL-12 and IFN- γ . On the other hand, the production of a Th2-type cytokine, IL-4, was drastically suppressed. Cytokine production was supported by the expression of mRNA in the draining lymph node. In terms of antigen-specific antibody production, the level of IgG2a which is regulated by IFN- γ was increased by CpG-ODN, but IgE production regulated by IL-4 was suppressed. Furthermore, administration of CpG-ODN via the skin drastically attenuated the production of IgE in mice undergoing IgE-type immune response. Administration of CpG-ODN through the skin may shift the immune response from Th2 to Th1-like response. These results suggested that administration of CpG-ODN via skin is a simple strategy for patients with diseases like AD, which is characterized by Th2-dominated inflammation.

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Keywords: CpG-ODN; Th1/Th2 balance; Transdermal administration; Atopic dermatitis

1. Introduction

Atopic disorders have a complex and chronic pathogenesis that provides many potential cellular and molecular targets for therapeutic intervention,

but may also include redundant pathways mediating disease [1,2]. CD4⁺ helper T (Th) cells have been subdivided into at least two subsets, Th1 and Th2, on the basis of their cytokine secretion [3,4]. The balance of Th1 and Th2 cells is relevant to the outcome of immunologically mediated clinical syndromes, and dysregulated Th1 or Th2 responses are thought to be central to the pathology of diseases

* Corresponding author. Fax: +81 426 76 3182.

E-mail address: aramaki@ps.toyaku.ac.jp (Y. Aramaki).

such as asthma and atopic dermatitis (AD), which is characterized by Th2-dominated allergic inflammation [5–7]. Th2-like immune responses mediated by the secretion of IL-4, IL-5 and IL-13 are key to the pathogenesis of atopic disorders such as allergic rhinitis, asthma, and AD. And it is also well known that AD is characterized clinically by cutaneous reactions resulting from barrier-disrupted skin, the skin being highly susceptible to antigen penetration. Penetrated antigen-specific responses in AD have been suggested to be dominated by a Th2 producing high levels of IL-4, IL-5 and IL-13. The ratio of Th2 to Th1 cells is increased in lesional skin of AD patients. On the other hand, Th1-like responses enhance cellular immunity associated with increased levels of Th1-like cytokines such as IL-2 and IFN- γ and cytotoxic T lymphocyte activity is up-regulated, indicating that the suppression of Th1 cell responses can result in the acceleration of tumor growth [8].

Unmethylated CpG dinucleotides flanked by certain bases (CpG-motif), which are present in bacterial DNA, have been shown to be immunostimulatory [9]. Previous studies have found that both the bacterial CpG-motif and synthetic oligodeoxynucleotides containing CpG-motif (CpG-ODN) activate cells such as B-cells, macrophages and dendritic cells, through toll-like receptor-9 (TLR-9) [9,10]. Signaling through TLR-9 leads to the secretion of large amounts of proinflammatory cytokines such as IL-1, IL-6, TNF- α , and IL-12 [11,12]. IL-12 acts on T and NK cells inducing the production of cytokines, primarily IFN- γ , enhancing NK cell cytotoxic activity and favoring the generation of cytotoxic CD8⁺ T lymphocytes. Furthermore, CpG-ODN rapidly activated B cells to proliferate and secrete IL-6 and IgM, and high levels of IFN- γ were produced by NK cells [13,14]. Consequently, CpG-ODN could be as an adjuvant for cellular and humoral immunities [15]. The induction of such innate immune responses and production of Th1-related cytokines is very important in controlling the early spread of pathogens and also in determining the type of antigen-specific immune responses that are induced [15]. Therefore, we expect the ability of CpG-ODN to shift the immune response from a Th2 to Th1-like response could exploit to develop an immunotherapy for patients with diseases like AD that are associated with a Th2-predominant immune

response [5–7]. Furthermore, the skin has recently become a target site for the development of non-invasive vaccine technologies [16,17].

In this study, we examined whether CpG-ODN alters the immune response to a model antigen, OVA, applied to tape-stripped skin in mice. CpG-ODN remarkably changed immune responses, causing a shift from a Th2 to Th1-type response as evaluated from cytokine and antibody levels. These results suggested that the skin is a potential site for CpG-immunotherapy in patients with AD and for vaccine development.

2. Materials and methods

2.1. Reagents

The sequences for CpG-ODN and non-CpG-ODN were 5'-TCCATGACGTTTCCTGATGCT-3' and 5'-TCCATGAGCTTCCTGAGTCT-3', respectively, and both HPLC-purified phosphorothioate ODNs were obtained from Amersham Pharmacia Biotech Co. (Tokyo, Japan). Rhodamine (Rho)-labeled CpG-ODN and Rho-non-CpG-ODN were also obtained.

2.2. Animal experiment

Balb/c mice (male, 6–8 weeks old) were purchased from Japan SLC Inc., (Shizuoka, Japan). Animal use and relevant experimental procedures were approved by the Tokyo University of Pharmacy and Life Science Committee on the Care and Use of Laboratory Animals. Mice were anesthetized with the i.p. injection of Nembutal (1.5 mg/mouse), the abdominal skin was shaved, and then the stratum corneum was removed by stripping with adhesive tape 6–10 times. After the application of FITC-labeled OVA (Sigma Chemicals) and Rho-labeled CpG-ODN onto the shaved skin, the skin was excised at specific points in time, embedded in Tissue-Tek OTC compound (Miles Inc., Elkhart, IN, USA), and snap-frozen in liquid nitrogen. The skin was then cut with a cryostat into 10- μ m vertical sections. To analyze the distribution of FITC-OVA and Rho-CpG-ODN, the dermal skin was fixed with 10% formalin at 4 °C for 16 h, embedded, snap-frozen, and cut as described above. The sections were examined by confocal laser scan-

ning microscopy (Micro-Radiance, Bio-Rad Laboratories Inc. Tokyo, Japan).

2.3. Preparation of mouse splenic cells

Following the application of CpG-ODN and OVA to the tape-stripped skin, the spleen was removed on day 5, and splenic single cell suspensions were prepared [18]. To examine the production of cytokines, spleen cells (5×10^5 /well) were incubated in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol for 24 h in the presence of OVA (2 mg/ml).

2.4. Cytokine determination

The levels of IL-4, IL-12 and IFN- γ in the supernatant of splenic cells were routinely determined with a sandwich ELISA using pairs of purified capture and biotinylated detection mAb (all obtained from Pharmingen) recognizing murine IL-4, IL-12 and IFN- γ , according to the manufacturer's protocols.

2.5. Changes in OVA specific-antibody levels

Blood samples from mice treated with OVA and CpG-ODN were collected on specific days from the retro-orbital plexus and sera were pooled. In brief, a doubling dilution of mouse sera (50 µl) were poured into an OVA-coated ELISA-plate, and stood overnight to confirm the immune reaction took place. Following

4 washes with PBS containing 0.05% Tween-20 (PBS-T), biotin-conjugated-anti-mouse IgG or IgE mAb (all obtained from Pharmingen), the plate was stood at 4 °C overnight. After further washing with PBS-T, avidin-conjugated peroxidase was added and the absorbance at 450 nm was determined as described previously [19].

2.6. RT-PCR

The changes in cytokine mRNA expression in lymphonode and skin were determined by RT-PCR. Total RNA (2 µg) was isolated with Isogen solution (Nippon Gene, Toyama, Japan) as reported previously [19]. cDNA was synthesized using SuperScript II. Then, cDNAs were amplified with primers specific for each cytokine. β -Actin primers were used as an internal control. Primers designed based on the mouse sequences were obtained from Amersham Pharmacia Biotech Co. (Tokyo, Japan), and the sequences are shown in Table 1. PCR was performed for a number of cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 60 s, and extension at 72 °C for 60 s. The amplified products were analyzed on 2% agarose gels containing 0.1 µg/ml of ethidium bromide.

2.7. Statistical analysis

The paired Student's *t* test was used to compare paired groups. Analysis of variance (ANOVA) was used for multi-group analysis. Values of $P > 0.05$ were considered to indicate lack of significance.

Table 1
Oligodeoxynucleotides used for PCR analysis in this study

Molecule	Primer	Sequence (5' to 3')	Product size	Cycles
IL-4	5'	AGTTGTCATCCTGCTCTTCTTT	344 bp	35
	3'	GACTGGGACTCATTCATGGTGC		
IL-5	5'	CAAAAAGAGAAGTGTGGCGAGG	703 bp	35
	3'	TAGATAGGAGCAGGAAGCCCCG		
IL-12 p40	5'	CAGAAGCTAACCATCTCCTGGTTTG	398 bp	35
	3'	TCCGGAGTAATTTGGTGGTTTCACAC		
IL-13	5'	GCAACGGCAGCATGGTATGGAG	173 bp	35
	3'	TGGTATAGGGGAGGCTGGAGAC		
IFN- γ	5'	CTCAAGTGGCATAGATGT	104 bp	30
	3'	GAGATAATCTGGCTCTGCAGGATT		
β -actin	5'	GCACCACACCTTCTACAATGAG	613 bp	30
	3'	TTGGCATAGAGGTCTTTACGGA		

3. Results

3.1. Localization of antigen and CpG-ODN

FITC-OVA and Rho-CpG-ODN were applied to tape-stripped mouse abdominal skin and the localization of OVA and CpG-ODN was examined by confocal laser scanning microscopy. In control skin (no tape-stripping), the fluorescent signals for FITC and Rho were observed faintly at the stratum corneum, suggesting that neither OVA nor CpG-ODN penetrated the subcutaneous layer (Fig. 1(a)). In the tape-stripped mice, however, green-fluorescence generated from FITC and red-fluorescence from Rho was observed around the corium at 1 h after the application (Fig. 1 (b)). Furthermore, after 12 h, the both fluorescences were overlapped partially at the corium (Fig.

1(c)), suggesting that OVA and CpG-ODN had penetrated and in some areas, co-localized in the skin.

3.2. Changes in cytokine levels in spleen cells

CpG-ODN induces the secretion of large amounts of proinflammatory cytokines such as IL-1, IL-6, TNF- α , and IL-12 from macrophages, dendritic cells and B cells [11,12]. IL-12 acts on T and NK cells inducing the production of cytokines, primarily IFN- γ . Furthermore, CpG-ODN has been reported to shift the immune response from a Th2 to Th1-like response [20–22]. Thus, the effect of CpG-ODN which was applied to the tape-stripped skin on the production of IFN- γ and IL-4 was investigated in vitro. Spleen cells were prepared from BALB/c mice on day 5 following the transdermal administration of

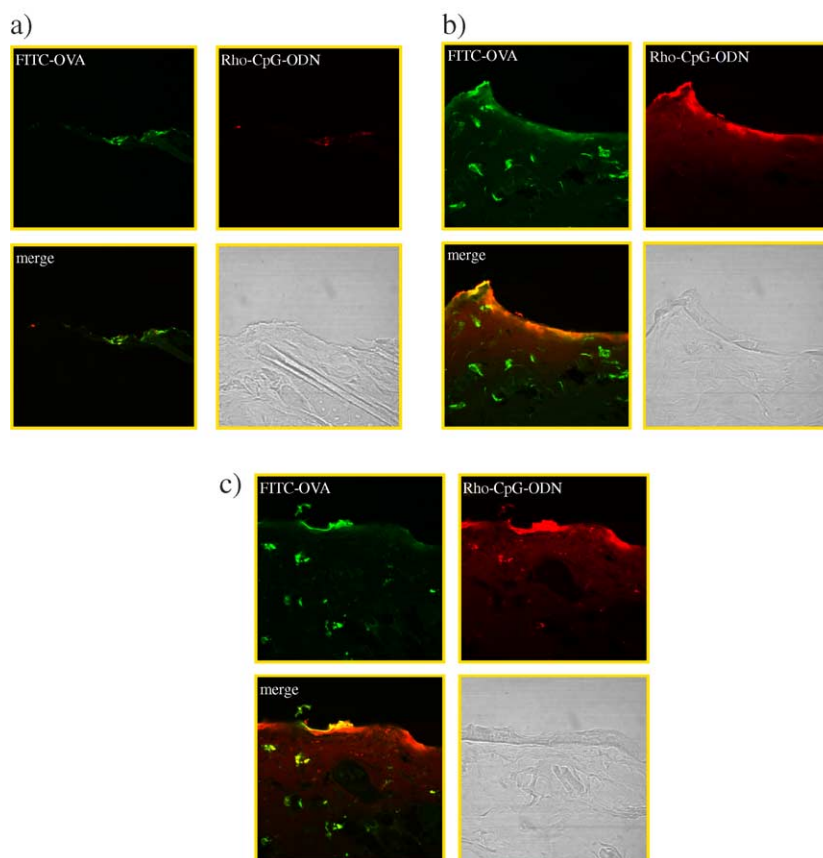


Fig. 1. Localization of FITC-labeled OVA and Rho-labeled CpG-ODN in Balb/c mouse skin following the topical administration. FITC-OVA (250 μ g) and Rho-CpG-ODN (50 μ g) were applied to the tape-stripped abdominal skin. (a); without tape-stripping, (b, c); stripped 10-times. Fluorescent images were obtained from same section at 1 h (a, b) or 12 h (c) after the application using confocal laser scanning microscopy.

OVA and CpG-ODN, and were incubated in the presence of 2 mg/ml of OVA for 72 h. Supernatants were collected and concentrations of IFN- γ and IL-4 were evaluated by ELISA. As shown in Fig. 2(a), the production of IFN- γ increased significantly on the co-application of OVA and CpG-ODN, and was 3-times that following treatment with OVA alone. On the other hand, the production of IL-4 by the spleen cells decreased drastically when the mice were treated with OVA and CpG-ODN, and was one forth the level in spleen cells from the mice treated with OVA alone (Fig. 2(b)).

The effects of CpG-ODN on the production of IFN- γ and IL-4 were dose-dependent. Furthermore, the effects of oligonucleotide having no CpG motif (Non-CpG-ODN) on the production of these cytokines were examined, and no influence on the production of these cytokines was observed (Fig. 3). These findings suggested that CpG-ODN applied to tape-stripped skin has the ability to change the immune responses to an antigen invading via the skin.

3.3. Effect of route of administration of CpG-ODN

To determine whether the route by which CpG-ODN is administered affects the immune response to

OVA applied to tape-stripped skin, the same amount of CpG-ODN was administered intravenously or intraperitoneally, or else applied to the skin. On day 5, the spleen was removed, splenic cells were prepared, and IFN- γ and IL-4 concentrations in the culture supernatant were evaluated by ELISA. In each case, levels of both cytokines increased significantly in comparison with the control, and the highest level of IFN- γ was observed in mice administered CpG-ODN through the skin (Fig. 4). The ratio of IFN- γ to IL-4, in other words the ratio of Th1-type to Th2-type cytokines, was about 5 and differed significantly. In spleen cells prepared from mice administered CpG-ODN intravenously, the ratio was 1.4 and showed no significant difference. In the intraperitoneally administered group, the level of IL-4 was higher than that of IFN- γ . These findings suggested that a more effective production of Th1-type cytokines occurs when CpG-ODN is administered via the same route as the antigen.

3.4. Changes in cytokine levels in the skin

It is reported that CpG-ODN promotes Th1-like immune responses and suppress Th2-like responses in vivo [20,21], thus examined the changes in cyto-

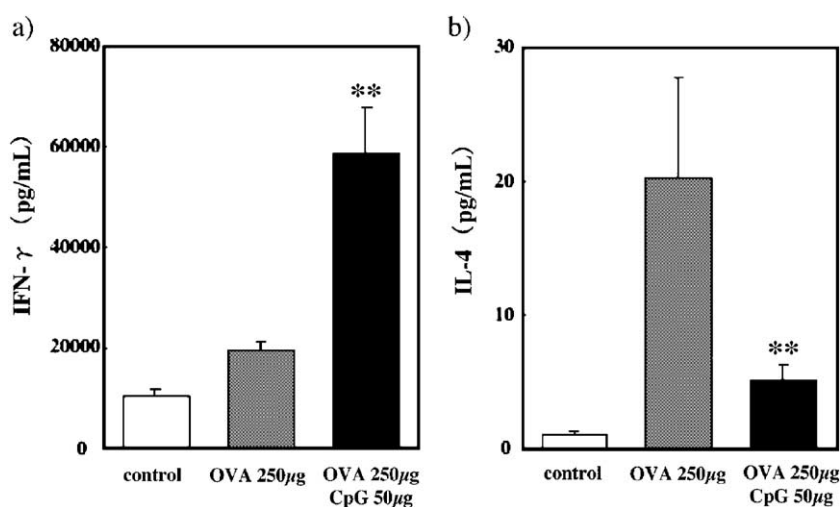


Fig. 2. Effect of CpG-ODN on the production of OVA-specific IFN- γ and IL-4 by spleen cells. Balb/c mice were sensitized to OVA and CpG-ODN through the skin. OVA (250 μg) and CpG-ODN (50 μg) were applied to shaved abdominal skin after barrier disruption by tape-stripping (10-times). On day 5, spleen cells (5×10^5 per well) were prepared and incubated in the presence of OVA (2 mg/ml) for 72 h. Concentrations of IFN- γ and IL-4 in the culture supernatants were evaluated by ELISA. Each value represents the mean $\pm$ s.e. for three mice. ** $P < 0.01$ compared with sensitization to OVA.

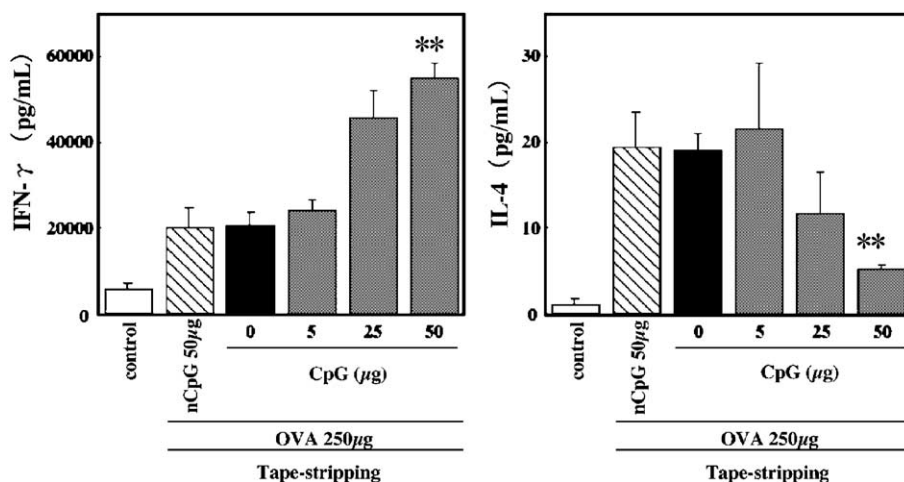


Fig. 3. Effect of CpG-ODN dose on the production of IFN- γ and IL-4 by spleen cells after sensitization with OVA and CpG-ODN through barrier-disrupted skin. On day 5, spleen cells (5×10^5 per well) were prepared and incubated in the presence of OVA (2 mg/ml) for 72 h. Concentrations of IFN- γ and IL-4 in the culture supernatants were measured by ELISA. Each value represents the mean $\pm$ s.e. for three mice. ** $P < 0.01$ compared with sensitization to OVA.

kinase levels in the skin 24 h after the application of OVA with CpG-ODN. In the tape-stripped skin, three cytokines (IL-12, IFN- γ , and IL-4) increased

without antigen or CpG-ODN, i.e., just tape-stripping (Fig. 5). IL-12 and IFN- γ levels increased significantly when OVA and CpG-ODN were

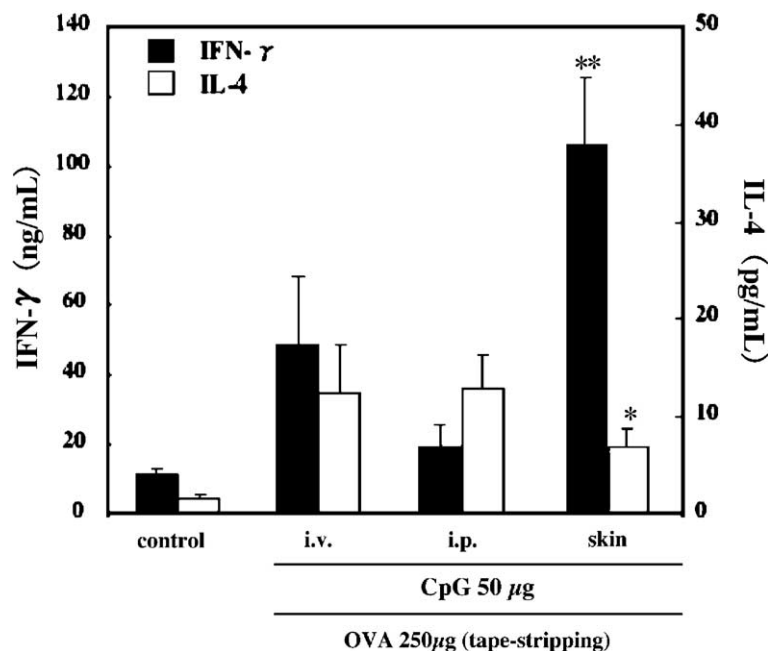


Fig. 4. Effect of administration pathway of CpG-ODN on the production of IFN- γ and IL-4 by spleen cells after sensitization with OVA. OVA (250 μ g) was applied to the tape-stripped skin (10-times) at the same time as CpG-ODN was administered by different routes; i.v., i.p. and via tape-stripped skin. Spleen cells (5×10^5 /well) were prepared 5 days after the application and incubated in the presence of OVA (2 mg/ml) for 72 h. IFN- γ and IL-4 productions in response to OVA were evaluated by ELISA. Each value represents the mean $\pm$ s.e. for three mice. ** $P < 0.01$, * $P < 0.05$ compared with i.v. and i.p. injection.

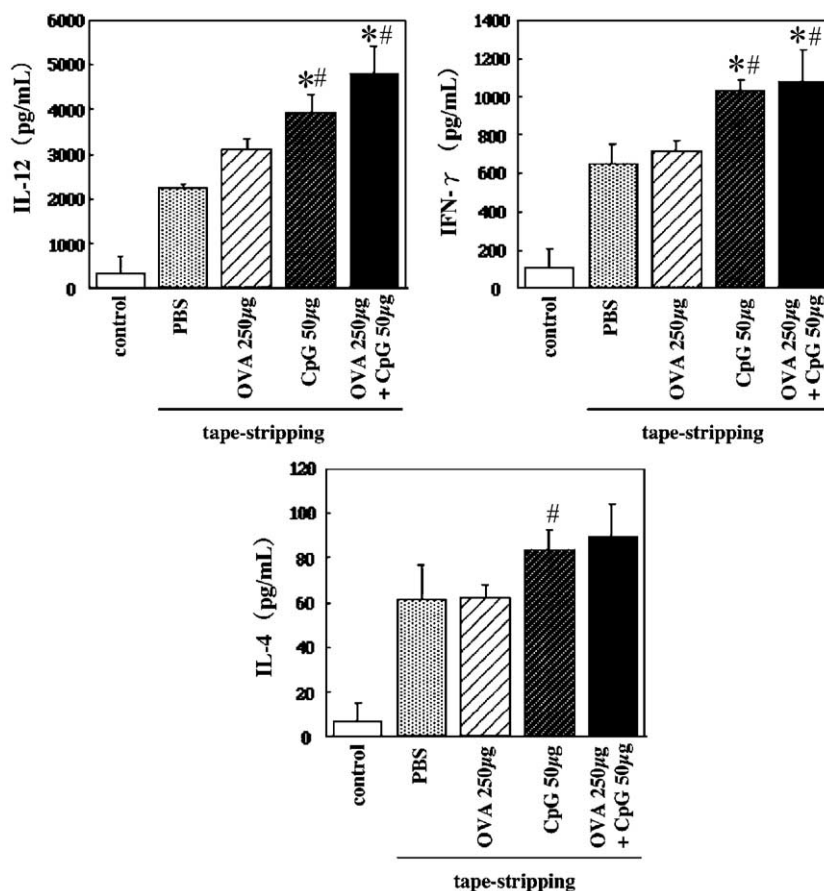


Fig. 5. Effect of CpG-ODN on IL-12, IFN- γ and IL-4 production in the skin. OVA and CpG-ODN were applied at 250 and 50 μ g to the tape-stripped abdominal skin and the levels of cytokines in the skin were determined 24 h following the administration. IL-12, IFN- γ and IL-4 concentrations were evaluated by ELISA. Each value represents the mean $\pm$ s.e. for three mice. * P < 0.05 compared with sensitization to PBS. # P < 0.05 compared with sensitization to OVA.

applied. However, no significant increase in IL-4 was observed even when OVA and CpG-ODN were co-administered.

Furthermore, mRNA levels of cytokines in the draining lymph node (LN) were evaluated by RT-PCR on day 4 following the application of OVA and CpG-ODN to the tape-stripped skin. As shown in Fig. 6, bands of IL-4, -5, and -13 mRNA were observed in the mice treated only with OVA via the skin, but no bands of IL-12 p40 and IFN- γ mRNA were observed, suggesting Balb/c mice showed a Th2-predominant immune response against OVA applied to tape-stripped skin. On the other hand, in the mice administered OVA and CpG-ODN, bands of IL-12 p40 and IFN- γ mRNA were clearly observed, whereas

the bands of IL-4, -5, and -13 mRNA became faint. These findings demonstrate that CpG-ODN changes the immune response to the antigen from the skin, to a Th1-dominant response.

3.5. Changes in antibody levels

As mentioned, CpG-ODN promotes Th1-like immune responses and suppresses Th2-responses *in vivo*. We therefore examined whether they redirected B cell differentiation toward a Th1-like phenotype [23], inducing multiple non-Th2-related isotypes. The changes in Ig isotypes are shown in Fig. 7. The production of IgG2a which is considered to be a Th1-like Ig isotype drastically increased when OVA was

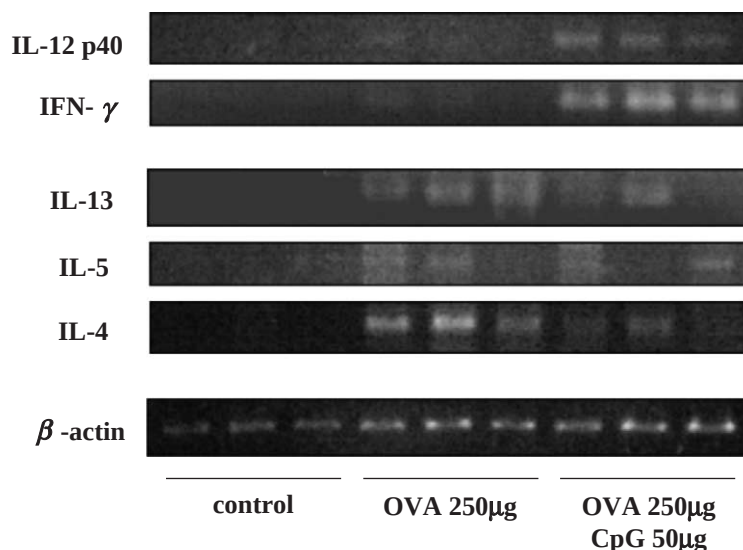


Fig. 6. Effect of CpG-ODN on cytokine mRNA expression after sensitization with OVA through the skin. OVA, and CpG-ODN were applied at 250 and 50 μ g to tape-stripped skin. Draining LNs were obtained 4 days after the application and the expression of cytokine mRNA was measured by RT-PCR analysis.

applied to the tape-stripped skin with CpG-ODN, but no production of IgG2a was observed in mice sensitized to OVA only. Furthermore, the production of IgE, a Th2-like isotype, was strikingly suppressed by the co-application of CpG-ODN (Fig. 7). These findings demonstrated that CpG-ODN co-applied with the antigen to tape-stripped skin changes the immune response from Th2 to Th1.

The regulation of IgE synthesis has been investigated extensively in recent years, and it has been found that IFN- γ and IL-4 strongly contribute to this regulation [24,25]. We next examined the effect of CpG-ODN on the production of IgE in mice which were already immunized with OVA and showed high levels of IgE. Following the application of OVA to the tape-stripped skin, CpG-ODN was administered twice

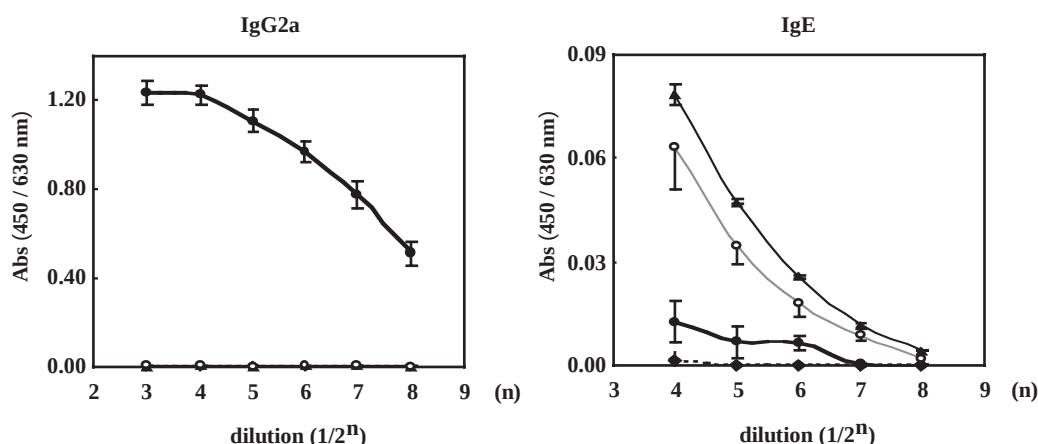


Fig. 7. Effect of CpG-ODN on OVA-specific antibody responses after sensitization with OVA and CpG-ODN through barrier-disrupted skin. OVA 250 μ g ($\blacktriangle$), OVA 250 μ g plus non-CpG-ODN 50 μ g (o) and OVA 250 μ g plus CpG-ODN 50 μ g ($\bullet$) were applied to the tape-stripped skin. Sera were collected 2 weeks after the application and OVA-specific IgG2a and IgE titers were evaluated by ELISA. Each value represents the mean $\pm$ s.e. for three mice.

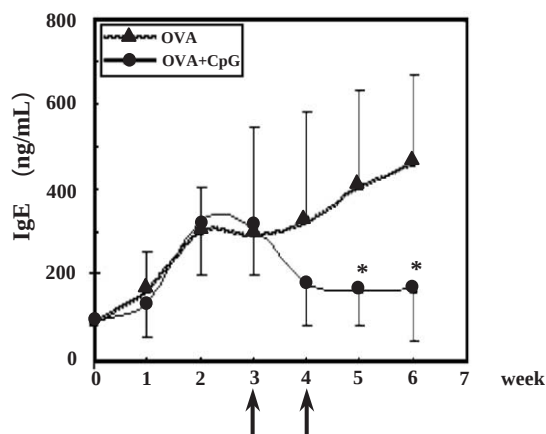


Fig. 8. Effect of CpG-ODN on the immune response in the mice ongoing IgE production. Mice were sensitized with OVA 20 μ g (▲) twice a week for 6 weeks through barrier-disrupted skin. The mice were then treated with CpG-ODN 50 μ g (●) twice at week-3 and -4. Sera were collected every week and the changes in IgE production were evaluated by ELISA. Each value represents the mean $\pm$ s.e. for five mice. Significant difference from the value in mice treated with a single sensitization (OVA), * $P < 0.05$.

3 and 4 weeks later at the time the mice showed high IgE levels. Then the changes in IgE levels in sera were evaluated by ELISA. As shown in Fig. 8, serum IgE levels increased gradually in the mice administered OVA only. On the other hand, in mice administered CpG-ODN, IgE levels decreased and a significant difference was observed 2 and 3 weeks following the administration of CpG-ODN. These results demonstrated that the administration of CpG-ODN via the skin attenuates drastically the production of IgE in mice undergoing an IgE immune response.

4. Discussion

CpG-ODN induces a Th1-like pattern of cytokine production that is dominated by IL-12 and IFN- γ with little secretion of Th2 cytokines, and then shifts the immune response from Th2 to Th1. We thus investigated whether topical application of CpG-ODN to tape-stripped skin changes the immune response from Th2 to Th1 by evaluating the production of cytokines and antibodies. First, we examined the localization of antigen and CpG-ODN following the application to tape-stripped skin. Since tape-stripping is a simple way to remove the epidermal barrier of the

stratum corneum [26], the model antigen, OVA with a molecular weight of 45,000 and CpG-ODN having high water solubility, easily penetrated the skin and fluorescence of FITC-OVA and Rho-CpG-ODN colocalized around the corium 12 h following the application (Fig. 1). CpG-ODN induces a Th1-like pattern of cytokine production that is dominated by IL-12 and IFN- γ with little secretion of Th2 cytokines, and then shifts the immune responses from Th2 to Th1. We thus investigated whether CpG-ODN alters the immune response to the topically administered antigen, and found a change from a Th2 to Th1-predominant response on the basis of the cytokines secreted (Fig. 2), expression of cytokine mRNA in LN (Fig. 6), and changes in the Ig isotype of sera (Fig. 7). It is well known that Th2-like immune responses mediated by the secretion of IL-4, IL-5 and IL-13 are key to the pathogenesis of AD [5–7]. However, significant change in IL-4 levels in the skin was not observed when OVA and CpG-ODN were co-administered (Fig. 5). Cytokine productions were examined 24 h after application of OVA and CpG-ODN, therefore antigen-specific T cell did not participate in this response. CpG-ODN could not change the immune response in the skin immediately after the application. But the ability of CpG-ODN, shift the immune responses from Th2 to Th1, in the skin lesion of AD may be achieved for long term therapy with CpG-ODN because Th1-predominant immune responses were observed in splenocyte (Fig. 2), lymph node (Fig. 6), and Ig isotype (Fig. 7).

Toll-like receptor-9 (TLR-9) is a receptor for CpG-ODN, and is known to be expressed in macrophages, B cells and DC [9,27]. Although keratinocytes have been shown to internalize ODN, they are not known to express TLR-9 [28]. Thus the Th1-predominant immune response may come from epidermal or dermal DC. On the other hand, the draining LN may contain a great number of cells that express TLR-9 compared to other tissues such as the skin and muscle, and concentrations of the CpG-ODN in the draining LN may be important as CpG-ODN shows biological activities. In the meanwhile, the Th1-predominant immune response may come from the DC or T cells which were activated as they migrated into LN. Recently, Lui et al. [28], reported that physical stress induces the expression of TLR-9 in the skin. The mechanism of the Th1-dominant immune response

of CpG-ODN against antigen in tape-stripped skin is still unclear.

Th2-type immune responses mediated by the secretion of IL-4, IL-5 and IL-13 are keys in the pathogenesis of atopic disorders [29]. The Balb/c mouse is known genetically to show a Th2-dominant immune response [30], and in this experiment, the mice showed Th2-dominant Ig isotype expression against OVA administered via tape-stripped skin (Fig. 7). Furthermore, the administration of CpG-ODN through the skin attenuated drastically the production of IgE in mice ongoing IgE immune responses (Fig. 8). The regulation of IgE synthesis has been extensively investigated in recent years, and it has been found that IFN- γ and IL-4 strongly contribute to this regulation [31–33]. IFN- γ and IL-4 promote IgG2a and IgG1 class switching, respectively [34,35]. Further, it has been reported that IL-4 and IFN- γ strongly regulate IgE synthesis, and the Th1-type cytokine IFN- γ acts as a counterpart of IL-4 and suppress the production of IgE. These findings suggested that IgE synthesis is highly dependent on the balance in the production of IL-4 and IFN- γ , and the difference in cytokine production following CpG-ODN administration affects the change in IgE production even in mice producing IgE.

Further, we examined the effect of the route of administration of CpG-ODN on immune responses. When CpG-ODN was administered with OVA via the same route, tape-stripped skin, Th1-type cytokine secretion was greater than an i.v. or i.p. administration. This may related to the findings that the direct conjugation of CpG-ODN to allergenic protein or peptides was more effective, possibly because of enhanced interaction with DC via the CpG-ODN [36,37], and suggested that CpG-ODN and OVA should be incorporated into the same DC to enhance the Th1-dominant immune response.

5. Conclusion

In conclusion, tape-stripping is a simple way to remove the epidermal barrier of the stratum corneum, and the skin is considered an excellent site for the non-invasive administration of a vaccine. Co-administration of CpG-ODN with OVA via tape-stripped skin was found to elicit an antigen-specific

Th1-predominant immune response by the evaluation of cytokine secretion and antibody production. Further, the application of CpG-ODN to the skin attenuates drastically the production of IgE in mice ongoing IgE immune response. Th2 responses are thought to be central to the pathology of diseases such as asthma and AD, which is characterized by Th2-dominated allergic inflammation. Administration of CpG-ODN through the skin may shift the immune response from Th2 to Th1-like response. These results suggested that administration of CpG-ODN via the skin is a simple strategy of immunotherapy for patients with diseases such as AD and tumors.

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