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Effect of Dendritic Cells Treated with CpG ODN on Atopic Dermatitis of Nc/Nga mice

Sang-Tae Park, Kyoung-Eun Kim, Kwangmin Na, Younghwa Kim and Tae-Yoon Kim*

Laboratory of Dermato-Immunology, Catholic Research Institute of Medical Science, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, South Korea

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Atopic dermatitis (AD) is a chronic inflammatory skin disease and the pathogenesis of AD is associated with the release of various cytokines/chemokines due to activated Th_2 immune responses. Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG dinucleotide in the context of particular base sequence (CpG motifs) are known to have the immunostimulatory activities in mice and to convert from Th_2 to Th_1 immune responses in AD. We aimed to investigate that CpG ODN, especially phosphodiester form, can stimulate the protective immunity in NC/Nga mice with AD. We isolated BMDCs from NC/Nga mice and then, cultured with GM-CSF and IL-4 for 6 days, and treated for 2 days by either phosphorothioate ODN or phosphodiester ODN. CpG ODN-treated DCs resulted in more production of IL-12. When CpG ODN-treated DCs were intravenously injected into the NC/Nga mice, the NC/Nga mice with CpG ODN-treated DCs showed significant improvement of AD symptoms and decrease of IgE level. Histopathologically, the NC/Nga mice skin with CpG ODN-treated DCs showed the decreased IL-4 and TARC expression comparing with non-injected mice. These results may suggest that phosphodiester CpG ODN-treated DCs might function as a potent adjuvant for AD in a mouse model.

Keywords: Atopic dermatitis (AD), IgE, IL-4, NC/Nga mice, Phosphodiester form

Introduction

Atopic dermatitis (AD) is one of the most common skin diseases and is characterized by pruritic and eczematous skin lesions (Yagi *et al*., 2002; Heishi *et al*., 2003). Recent progresses on the pathogenesis of AD have revealed that it is associated with a variety of immunological abnormalities, such as the elevation of IL-4, IFN-gamma and IL-10 responses (Nakazawa *et al*., 1997; Lebwohl, 1998; Akidis *et al*., 1999; Akidis *et al*., 2000; Yagi *et al*., 2002) and immunoglobulin E (IgE)-mediated antigen presentation of (aero-) allergens has been considered a key event in the pathogenesis of AD (Reinhold *et al*., 1998; Wollenberg and Bieber, 2000). Patients with AD show elevated plasma IgE levels against many kinds of allergens (Sasakawa, 2001) and keratinocytes of patients with AD exhibit a propensity to exaggerated production of cytokines and chemokines, a phenomenon that can have a major role in promoting and maintaining inflammation (Nakazawa *et al*., 1997; Wohllenben and Erb, 2001). Immunomodulation therapy offers a possible treatment modality for AD, but currently, there is no specific therapeutics available.

The recent advances in immunology has focused on dendritic cells (DCs) as they are the key regulators of the adaptive immune system (Matera *et al*., 2001), are also unique players in priming naïve T cells and can produce Th-polarizing cytokine (Luster, 2002). DCs originate from pluripotent stem cells in the bone marrow, enter the blood stream and localize into various organs (Moser and Murphy, 2000). DCs can direct different types of T cell responses. So far, the DC-mediated immunotherapy or therapeutic vaccine approach has been investigated in diseases such as cancer and infectious diseases (Sato *et al*., 2003; Santini and Belardelli, 2003). Microbial stimuli, cytokines, chemokines, and T cell-derived signals have been shown to trigger cytokine synthesis by DCs (Edwards *et al*., 2002). IL-12 production by DC stimulated by microbial stimuli has been shown to drive Th1 responses (Jakob *et al*., 1999). In this regard, CpG-containing ODN was reported to activate DC to prime Th1 response *in vivo*, resulting in protection from *L.major* infection (Ramirez-Pineda *et al*., 2004).

NC/Nga mice were originated from Japanese fancy mice in 1957 by Kondo (Nagoya University, Nagoya, Japan). They develop AD-like skin lesion and IgE hyperproduction under

*To whom correspondence should be addressed.
Tel: 82-2-590-1341; Fax: 82-2-3482-8261
E-mail: tykimder@catholic.ac.kr

conventional conditions with itching, erythema and hemorrhage, followed by edema superficial erosion, deep excoriation, scaling, dryness of the skin and retarded growth (Matsuda *et al*., 1997; Vestergaard *et al*., 2000). These pathophysiological observations in AD of NC/Nga mice highly resemble those in human AD, so this strain of mouse has been considered a useful animal model to study pathologic mechanisms of human AD (Suto *et al*., 1999). Thus, the use of CpG-ODN-activated DCs to shift Th1/Th2 balance to Th1 type in NC/Nga mice provides an appropriate animal system to test the possible immunotherapy to treat AD in humans.

In the present study, we investigated the possibility of DCs activated by CpG ODNs to prime Th1 response in an animal model of AD, NC/Nga mice system, aiming to improve AD symptoms. Previous attempts to identify active phosphodiester CpG ODN with immunostimulatory activity have revealed one candidate (46-O) which was demonstrated to be equivalent to phosphorothioate-ODN (1826-S) in terms of suppressing Th2 responses in NC/Nga mice (Choi *et al*., 2006). In this investigation, we compared the efficacy of the 46-O to that of 1826-S in activating DC to prime Th1 response while suppressing Th2 response in NC/Nga AD mouse model, thus in improving AD symptoms.

Materials and Methods

Animals. Six week-old female NC/Nga mice were purchased from SLC (Shizuoka) and were maintained for 2 weeks before the start of the experiments. They were housed in an air-conditioned animal room with a 12 h light/dark cycle (08 : 00-20 : 00 h light, 20 : 00-08 : 00 h dark), at 23 ± 2°C and a humidity of 50 ± 10%. Mice were provided with a laboratory diet and water ad libitum. The study was approved by the Institutional Animal Care and Use Committee of Catholic University and all procedures were conducted in accordance with the U.S. National Institutes of Health guidelines.

Reagents. The sequences of the CpG ODNs (synthesized by Genotech corp., Daejeon, Korea) used were and are as follows: 1826-S, 5'-TCCATGACGTTCCTGACGTT-3'; and 46-O, 5'-CTCG CACGTTGCCGACTTC-3. LPS (Sigma) was used at a concentration of 100 ng/mL. Anti-CD80, anti-CD86 and MHC class II-anti-I-A^d antibodies were from purchased from Pharmingen (San Diego, CA, USA). Recombinant murine GM-CSF and IL-4 were purchased from Biosource (Camarillo).

Isolation of DCs from bone marrow cultures. Bone marrow-derived DCs (BMDCs) were generated from culture of bone marrow cells in tibial and femoral bones of NC/Nga mice as described previously (Scheicher *et al*., 1992; Lutz *et al*., 1999). The cells (1×10^6 cells/mL) were seeded onto a six-well culture plate in complete RPMI-1640 medium supplemented with recombinant murine GM-CSF (10 ng/mL) and IL-4 (10 ng/mL). The culture medium was changed every 2 days to remove granulocytes. BMDCs were pulsed with LPS or CpG ODNs (1826-S or 46-O) on day 6 of culture. Loosely adherent clustering cells were used as immature DCs for further experiments.

Flow cytometric analysis of DC preparations. Flow cytometric analysis was performed as described earlier (Ni, 2000; Rodriguez *et al*., 2005). DC was subcultured in GM-CSF and IL-4 supplemented media for 24 h in six-well plates in the presence or absence of LPS, 1826-S or 46-O. Data were acquired on a Becton Dickinson FACScalibur (Becton Dickinson) equipped with two lasers (excitation at 488 nm and 635 nm wavelengths) and were analyzed using CellQuest software (Becton Dickinson). Propidium iodide-permeable cells were excluded from analyses.

Quantitative RT-PCR. RT-PCR was performed using total RNA isolated from the cell by TRIzol reagent (Gibco-BRL), according to the manufacturer's instruction, and reverse-transcribed into cDNA using M-MLV RT 200 U/20 μL reaction volume, RNase inhibitor 40 U/2 μL reaction volume, and 3 μg of total RNA. One microlitter of the resulting cDNA was amplified by PCR using primers specific for IL-12p40 cDNA. The sequences of primers used are: IL-12p40, forward; 5'-CCTAGGATGCAACGTTGGAAAG-3' and reverse; 5'-ACAGCTTCTTCATGTCTCCAA-3. PCR-amplified material was separated on 1% agarose gels and visualized under UV light after ethidium bromide staining.

IL-12 detection. DC was suspended in GM-CSF and IL-4 containing media and incubated for indicated in the presence or the absence of LPS, 1826-S or 46-O. At the end of incubation, supernatants were decanted, non-adherent cells were removed by centrifugation, and the cell-free supernatants were stored at −70°C until use. IL-12 in the culture supernatant was measured using Quantikine ELISA kit specific to mouse IL-12p40 from Biosource (Camarillo).

Measurement of serum IgE. After anesthetizing the NC/Nga mice with diethylether, blood sample was collected from the eye. The blood was then spun at 3,000 rpm for 10 min to collect serum. Serum IgE level was measured using BD OptEIA kit (BD pharmingen).

Injection of NC/Nga mice with DC. NC/Nga mice were immunized with unpulsed DC or DC pulsed with either 1826-S or 46-O. Pulsing with CpG ODN for 48 h was performed as described previously (Du *et al*., 2006). CpG ODN-pulsed or unpulsed DCs were washed with PBS followed by tail *i.v.* injection (210^5 cells/mouse in 100 μL of PBS) into NC/Nga mice at the age of 12 weeks. Mice in all groups were sacrificed 5 days after receiving the last treatment and skin, blood and spleen were isolated.

Histology. Tissue samples were stained with hematoxylin-eosin (H&E) for gross histological examination, with toluidine blue (TB) for detection of mast cells (Nakamura *et al*., 2002), with anti-IL-4 or with anti-TARC antibodies using standard procedures (Matsuda *et al*., 1997; Kakinuma *et al*., 2001). Briefly, tissue samples of skin from the back of each animal were fixed in 4% formalin solution overnight and embedded in paraffin by the conventional method, cut into 4-μm thick sections. Deparaffinized sections were treated for 15 min with 3% hydrogen peroxide in phosphate-buffered saline (PBS). After blocking in 10% normal goat serum diluted in PBS with 0.1% bovine serum albumin (BSA), sections were incubated overnight at 4°C with the primary antibody. Also, other

skin biopsies were embedded in O.C.T. medium (Tissue-Tek; Sakura Finetek), snap-frozen immediately in liquid nitrogen and stored at −80°C until use. For histology and immunohistochemistry, 4-μm thin cryosections were prepared, dried in air and fixed in acetone for 10 min. Sections were incubated with the specific antibody. After staining with the primary antibody, the samples were incubated with the biotin-labeled secondary antibody and the staining was visualized with DAB Substrate chromogen system (DAKO, Denmark).

Statistical analysis. Data were expressed as the mean ± SEM. Statistical significances were assessed by Student's t-test and $p < 0.05$ was taken as the level of significance.

Results

Expression of MHC and co-stimulatory molecules on BMDC after stimulation with CpG ODNs. Mouse primary DCs are known to be activated by several microbial stimuli, such as LPS and CpG ODNs *in vitro*. BMDCs prepared from NC/Nga mice were incubated with test stimuli for 48 h, and expression of activation markers, MHC-II, CD80 and CD86 was measured by FACS. Medium alone or LPS (100 ng/mL) was included as negative or positive control, respectively. The sequence of each of the CpG ODNs, 1826-S or 46-O, is described in Materials and Methods. As shown in Fig. 1A, treatment of DC with 4 μg/mL of 1826-S led to significantly increased expression of MHC-II and CD80, from 48% and 27% of control to 76% and 47%, respectively. The expression of CD86, however, was only slightly enhanced over the control of medium alone when treated with 4 μg/mL of 1826-S, but the increase was still statistically significant. Thus, for further experiments, 1826-S was used at 4 μg/mL. Meanwhile, 46-O at 4 μg/mL, did not increase expression of any of the markers, MHCII, CD80 or CD86. Since identical concentration of 46-O (4 μg/mL) did not induce any significant changes in cell surface phenotypes as observed with 1826-S, we tested the DC-stimulating activity of 46-O at higher concentrations, up to 50 μg/mL, and chose 16 μg/mL for further analyses (data not shown). Upon stimulating with DC at 16 μg/mL of 46-O, the expression of MHC-II and CD80 on DC significantly increased. However, the expression of CD86 on DC did not increase (Fig. 1B). This result demonstrated DC-stimulating activity of 1826-S and the activated DC was further analyzed

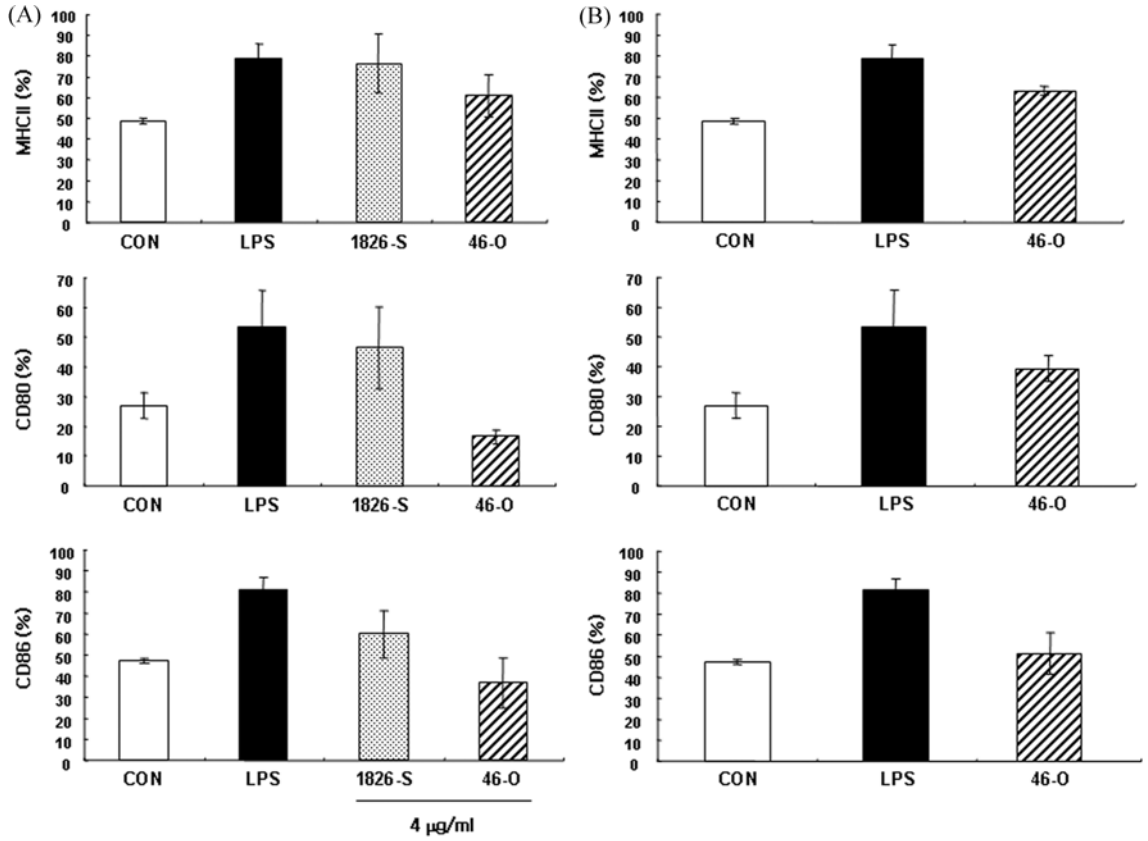

Fig. 1. Enhanced expression of MHC and costimulator molecules by bone marrow-derived dendritic cells (BMDCs) treated with CpG ODNs. BMDCs were cultured for 6 days with GM-CSF (10 ng mL^{-1}) and IL-4 (10 ng mL^{-1}), and then cells were treated CpG ODNs. BMDCs were cultured 48 h in the absence (control) or presence of LPS, 1826-S or 46-O and activity was analyzed with flow cytometry. Numbers indicate the mean fluorescence intensity of staining with the different monoclonal antibodies after subtraction of backgroud from isotype-matched controls. Representative data from one of three (n = 3) experiments are shown.

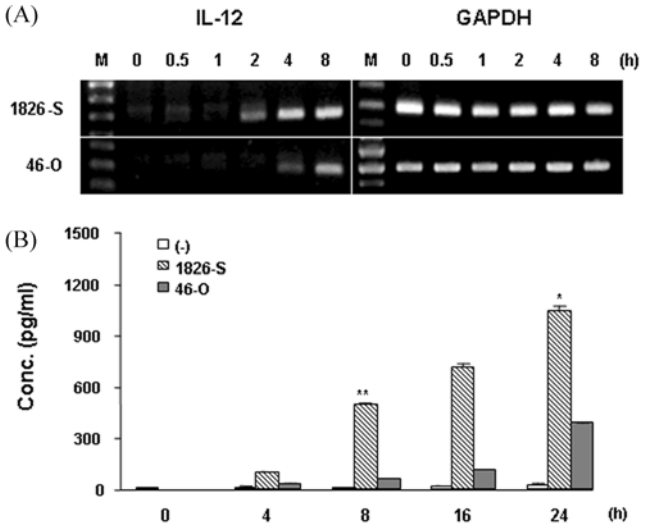

Fig. 2. Cytokine production by BMDC from NC/Nga mice after incubation with GM-CSF (10 ng mL^{-1}), IL-4 (10 ng mL^{-1}). BMDC were stimulated with CpG ODNs. (a) Reverse transcriptase (RT)-PCR analysis of IL-12 mRNA was performed on BMDCs grown in GM-CSF, IL-4 for 6 days. The dose of 1826-S and 46-O was from 4 to 16 μg mL^{-1}, respectively. (b) The content of IL-12p40 in the supernatant was determined by ELISA. Values were expressed as mean ± SEM. Significantly different from the control, *; $p < 0.05$, **; $p < 0.01$.

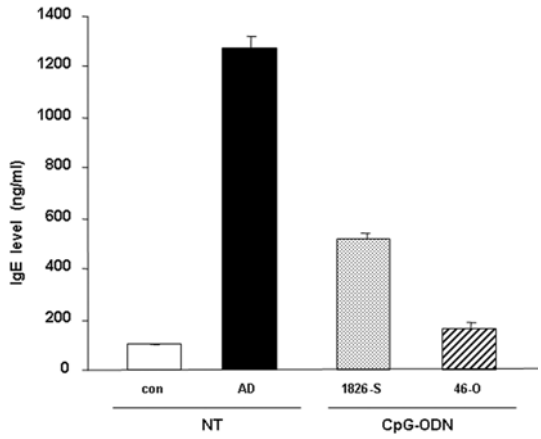

Fig. 3. Decrease of serum IgE level by BMDC after stimulation with CpG ODNs. BMDC after stimulation with CpG ODNs was *i.v.* injected (2×10^5 cells mL^{-1}) in AD of NC/Nga mice at 12 weeks of age, and the levels of IgE were determined 5 days after the *i.v.* injection. NC/Nga mice were non-treated (NT) or were treated with BMDC after stimulation with CpG ODNs. Each value represents the mean ± SEM for three mice in each group.

for its efficacy in suppression of Th2 response in following experiments.

Activation of DC by CpG ODN treatment. The ability of DC to release IL-12 in response to microbial stimuli is regarded as critical for induction of Th1 response (Moser and Murphy, 2000). Thus, in order to assess CpG ODN pulsed DC's ability to induce Th1 polarization, expression of IL-12 in CpG ODN-treated DC was measured by RT-PCR using total RNA and ELISA using culture supernatant. As shown in Fig. 2A, the expression of IL-12 increased gradually with prolonged incubation with either 1826-S (4 μg/mL) or 46-O (16 μg/mL). Interestingly, the expression of IL-12 was detectable at 2 h of treatment with 1826-S, and was reaching plateau at 8 h of treatment, whereas 46-O stimulation demonstrated somewhat delayed induction of IL-12 expression compared to that of 1826-S. The expression of IL-12 in DC didn't result in significant changes when DC were treated with control ODNs (data not shown). Production and release of IL-12 by DC was further investigated by ELISA as shown in the Fig. 2B. In consistent with Fig. 2A, 1826-S-pulsed DC started showing significantly increased production of IL-12 upon 8 h of treatment, which gradually increase with prolonged incubation. In addition, 46-O did not induce production of IL-12 as strongly as 1826-S, albeit significant IL-12 was shown to be released. Furthermore, control ODNs did not induce any increase in IL-12 release from DC (data not shown). Thus, 1826-S was shown to be a potent inducer of IL-12 production from DC, implying its role in Th1 polarization.

Decrease of serum IgE level by BMDC after stimulation with CpG-ODNs. We next examined the ability of CpG ODN-pulsed DC to improve AD symptoms when injected into NC/Nga mice. AD is characterized by increased serum IgE level, in addition to, marked pruritic and eczematous skin lesions. The serum IgE level of NC/Nga mice with AD was reported to be more than 3-fold greater than that of healthy control mice (Takakura *et al*., 2005). Thus, we investigated if DC stimulated with either 1826-S or 46-O were able to reduce the serum IgE level in NC/Nga mice. After pulsed with either type of CpG ODN, DC were injected into NC/Nga mice (2×10^5 cells/mL) at 11~12 weeks of age with AD symptoms, and the level of the serum IgE was measured on day 5 post injection. As demonstrated in the Fig. 3, injection of 1826-S-pulsed DC strongly decreased serum IgE level to approximately 500 ng/ml (compare 1,300 ng/mL in untreated mice with AD symptoms). Surprisingly, injection of DC pulsed with 46-O, decreased the serum IgE more significantly than 1826-S, showing reduction of the IgE level to approx 200 ng/mL. The experiments were repeated multiple times and the similar results were obtained. Considering the previous observation which revealed the 1826-S was more effective in activating DC as measured by increase in the expression of MHC-II, CD80 and CD86 as in addition to production of IL-12, this was rather unexpected data. However, in the study where 1826-S or 46-O was topically applied to the AD lesion of NC/Nga mice with AD symptoms, we also found further decrease of the serum IgE with 46-O (unpublished data). Although speculative at this moment, we hypothesize that there is likely to be an optimum level of DC activation to yield the most reduction of the serum IgE in NC/Nga mice model.

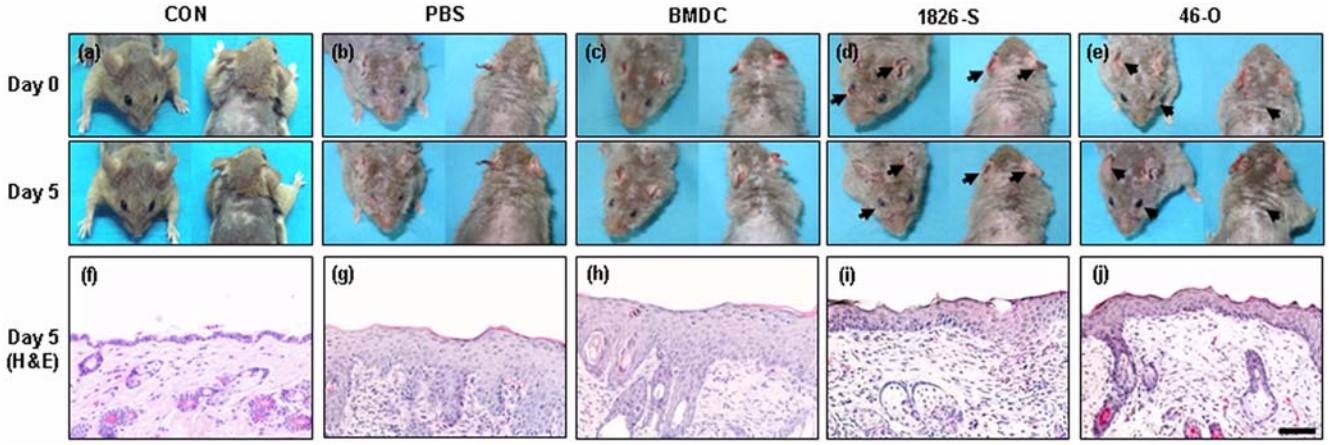

Fig. 4. Clinical observations of the skin lesions after *i.v.* injection of BMDC (2×10^5 cells mL^{-1}) after stimulation with CpG ODNs was *i.v.* injected at day 0 in AD of NC/Nga mice at 12 weeks of age. Day 0, before the injection of BMDC after stimulation with CpG-ODNs; day 5, after the injection of BMDC after stimulation with CpG ODNs. (a); SPF control, (b); Only injected with PBS, (c); Injected with BMDC without CpG ODN, (d); Injected with BMDC after stimulation with 1826-S, (e); Injected with BMDC after stimulation with 46-O. Hematoxylin and eosin staining after injection of BMDC after stimulation with CpG ODN. At 5 day after the injection, the skin was resected, cut into 4-μm sections, stained, and examined by light microscopy for histopathological changes (f~j). The results shown are representative of three mice in each group. Scale Bar = 60 μm.

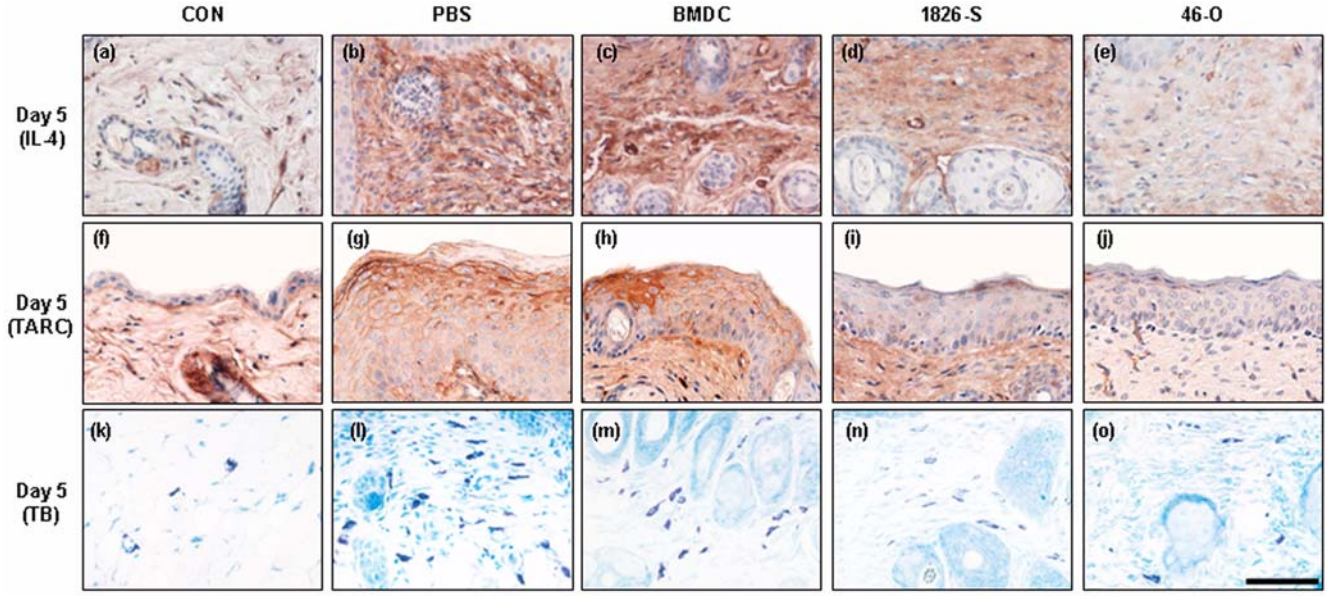

Fig. 5. Immunohistochemical staining of paraffin sections of skin biopsies. IL-4, TARC and toluidine blue staining of biopsies taken from 12 week mice kept in conventional conditions. IL-4, TARC and toluidine blue staining of skin, (a, f, k); SPF control, (b, g, i); Only injected with PBS, (c, h, m); Injected with BMDC without CpG ODN, (d, i ,n); Injected with BMDC after stimulation with 1826-S, (e, j, o); Injected with BMDC after stimulation with 46-O. Scale Bar = 30 μm

Effect of DC stimulated with CpG ODN on skin lesion of NC/Nga mice with AD. We next examined the lesional skin of NC/Nga mice after injecting with PBS only, unstimulated DC or DC pulsed with CpG ODN. Gross clinical observation demonstrated that the mice injected with PBS only or unstimulated DC showed persistent, severe dermatitis, and scratching behavior during the treatment from day 0 (before injection) until day 5 (Fig. 5 (b) and (c)). In contrast, the mice injected with DC pulsed with either 1826-S or 46-O showed AD symptoms on day 0 and this almost disappeared on day 5 post-injection (Fig. 5 (d) and (e)). Histopathological investigation of the affected skin lesion was performed on paraffin-embedded skin section of the mice (Fig. 5 (f) through (j)). The mice injected with CpG ODN-pulsed DC, on day 5, show remarkable improvement in AD symptoms, displaying amelioration of hyperkeratosis, acanthosis, and hypergranulosis,

which is in consistent with gross clinical observation (Fig. 5 (b) through (e)). These data suggest that DC pulsed with either 1826-S or 46-O was able to reduce AD symptoms in NC/Nga mice. We found the decreased expression of IL-4 and TARC which are known markers for AD and reduced infiltration of mast cells in skin lesions of NC/Nga mice with CpG ODN-pulsed DC (Vestergarrd *et al*., 2000; Song *et al*., 2006; Saeki and Tamaki, 2006). As shown in the Fig. 5 (b) and (c), strong immunoreactivities of IL-4 was detected in AD-lesions obtained from NC/Nga mice either injected with PBS alone or unpulsed DC, which is in consistent with persistent AD symptoms observed under the same conditions. However, dramatic reduction of IL-4 expression was shown in skin lesion of mice on day 5 post-injection with either 1826-S or 46-O (Fig. 5 (d) and (e)). Despite the difference in lowering the serum IgE level between 1826-S and 46-O, we did not find any differential expression of IL-4 between those two groups.

Level of TARC expression was examined and the results are shown in the Fig. 5 (f) through (j). Similar to IL-4, expression of TARC was found to be elevated in AD lesion of NC/Nga mice injected with PBS alone or with unpulsed DC (Fig. 5 (g) and (h)). Injection of NC/Nga mice with CpG ODN-pulsed DC led to reduction of TARC expression in skin lesion (Fig. 5 (i) and (j)). Interestingly, 1826-S-pulsed DC induced decrease of epidermal TARC, whereas both epidermal and dermal TARC expression was found to be lowered by injection of 46-O-pulsed DC. Next, we investigated the number of infiltrating mast cells in skin lesions of NC/Nga mice by staining with toluidine blue (TB). In accordance with improvement of AD symptoms in NC/Nga mice, injection of CpG ODN-pulsed DC led to reduction of mast cells which otherwise infiltrate into the AD lesions (compare Fig. 5 (l) and (m) vs. (n) and (o)), suggesting amelioration of inflammatory responses. These data suggest that the reduction of IL-4 and TARC expression by injection of CpG ODN-pulsed DC was responsible, at least in part, for improvement of AD symptoms in NC/Nga mice, implying therapeutic potential of this DC-based approach for AD in humans.

Discussion

In this study, we investigated potential application of CpG ODN-activated DC in treatment of AD using NC/Nga mice system. DC are the most potent antigen presenting cells and are capable of initiating primary and memory T cell responses (Homey *et al*., 2006). Upon stimulation, DC releases IL-12 which is considered to be a switch for induction of Th1 responses (Moser and Murphy, 2000). Due to its capability to induce Th1 response, activated DC-based therapeutic approach has been tested in several clinical applications, especially in eradication of tumors and protection from infection (Brunner *et al*., 2000; Sato *et al*., 2003; Ramirez-Pineda *et al*., 2004; Pilon-Thomas *et al*., 2006). Microbial stimuli, cytokines, chemokines, and T cell-derived signals all have been shown to trigger Th-polarizing cytokine synthesis by DC (Edwards *et al*., 2002).

The NC/Nga mice have been proposed as a model of human AD, which is described as a Th2-type disease at least in the initiating phase (Takano *et al*., 2005). These mice start developing AD symptoms with skin scratching 8 weeks after birth when mice are raised in conventional rearing conditions. AD lesions first appear on the head and back of the neck at 10 weeks, spread to the back and ears at 11 weeks, spread over the whole body at 13 weeks, and reach a plateau at 17 weeks after birth (Matsuda *et al*., 1997). Histopathological examination of AD lesions in conventional NC/Nga mice showed the typical features of affected skin observed in human AD patients, such as an increased number of mast cells with marked degranulation (Groneberg *et al*., 2005). The disease developing NC/Nga mice revealed predominant Th2 responses and elevated serum IgE levels. The eliciting factor of the dermatitis is not known, but when BALB/C mice were kept under conventional conditions with the NC/Nga mice, they did not develop any lesions, indicating that a genetic factor in addition to an environmental factor is responsible for the development of the dermatitis.

The data presented in this work showed that CpG ODN stimulated DC, as expected, showing increased surface expression of MHC-II, CD80 and CD86 as well as IL-12 production, suggesting a switch to Th1 response. Furthermore, the stimulated DC were capable of improving AD symptoms in NC/Nga mice. DC can mature by various stimuli such as ligation of pattern-recognition receptors, pro-inflammatory cytokines, necrotic cells or only cluster disruption (Voigtlander *et al*., 2006). As a result, DC underwent complex changes including upregulation of MHC-II and costimulatory molecules. Indeed, in our experiments, DC derived from bone marrow of NC/Nga mice showed increased surface expression of MHC-II, CD80, and CD86 molecules in response to CpG-ODN treatment.

Among microbial stimuli that can activate DC, CpG-containing ODN, the sequences of which were derived from bacterial genomic DNA, was shown to directly activate DC via Toll-like receptor 9 (TLR9) (Hemmi *et al*., 2000; Wang *et al*., 2005). For most application with CpG ODN, phosphorothioate-modified ODN are used due to their resistance to nucleases, thus stability against degradation (Stein *et al*., 1988). Phosphodiester-ODN, however, are degraded rapidly inside eukaryotic cells (Zhao *et al*., 1993).

Several studies have reported elevated serum IgE level in NC/Nga mice with AD-like skin lesions (Sakamoto *et al*., 2004; Takakura *et al*., 2005). In the present study, total IgE level in the groups injected with 46-O-pulsed DC were significantly lower than those with 1826-S-pulsed DC although 1826-S was more capable of stimulating DC as indicated by further increase in expression of MHC-II, CD80 and CD86. Although the mechanism underlying this observation is yet to be investigated, we speculate there exist an optimum level of

DC stimulation which could induce proper Th1/Th2 switching *in vivo*, leading to improvement of AD symptoms.

In general, IgE synthesis by B cells is primarily regulated by cytokines (Renz *et al*., 1994). Th2 cytokines such as IL-4 play a key role in the hyperproduction of IgE, whereas Th1 cytokines, especially IFN-gamma, are strong inhibitors of IgE synthesis, Th2 cell proliferation and IL-4 receptor expression on T cells (King *et al*., 1989; Matsumoto *et al*., 1999). The expression of IL-4 decreased in skin lesion of the mice injected with CpG ODN-pulsed DC compared to that from control mice. These results imply that CpG ODN-pulsed DC injection can reduce the AD symptom by suppressing the Th2 cell response. Along with IL-4, TARC is a well-known marker for AD. Upon examination of TARC expression in AD lesion, it was revealed that the production of TARC was localized mainly to the basal layer of the epidermis. On the other hand, the mice injected with CpG ODN-pulsed DC showed lowered expression of TARC. Especially, 46-O was more capable of inducing decrease in TARC expression and the mechanism for this is unknown.

In conclusion, stimulation of DC with CpG ODN (1826-S or 46-O) led to increased IL-12 production in addition to upregulation of CD80, CD86 and MHC-II. The dosage of each CpG ODN, however, was different, showing requirement for higher dose of 46-O to activate DC. Upon injection of NC/Nga mice with CpG ODN-pulsed DC, improvement of AD symptoms was detected, suggesting suppression of Th2 response as indicated by decrease of IL-4 and TARC expression. Based on the observation with AD mice model, we suggest that the CpG ODN-mediated DC stimulation may become an alternative therapeutic modality for treatment of Th2-dominant immunological disorders such as AD.

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