

IL-4/IL-13 antagonist DNA vaccination successfully suppresses Th2 type chronic dermatitis

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Summary

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Conflicts of interest

None declared.

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Background Atopic dermatitis (AD) is a chronic disease with a Th2-type-cytokine dominant profile. Several cytokines and related peptides have been used for the treatment of AD but they were ineffective because of their limited biological half-life. We have recently developed a highly efficient mouse dominant negative interleukin (IL)-4/IL-13 antagonist (IL-4DM), which blocks both IL-4 and IL-13 signal transductions.

Objective To examine the effects of IL-4DM *in vivo* in an AD model induced by the repeated exhibition of oxazolone (OX).

Methods Plasmid DNA was injected intraperitoneally to cause an experimental AD-like dermatitis. The effect was evaluated by ear thickness, histological findings, and mast cells counts in the inflamed skin. The plasma IgE and histamine levels were measured. Cytokine production in skin and splenocytes were also analysed.

Results Mice treated with control plasmid developed marked dermatitis with mast cells and eosinophil infiltration, and had increased plasma IgE and histamine levels with a Th2 type splenocyte cytokine profile. Treatment with mouse IL-4 DNA augmented the ear swelling and thickness with an increased dermal eosinophil count, plasma histamine level, and production of splenocyte IL-4. However, IL-4DM treatment successfully controlled the dermatitis, decreased the mast cell and eosinophil count, and suppressed plasma IgE and histamine levels. Splenocytes produced an increased level of IFN- γ .

Conclusion These data showed that the simultaneous suppression of IL-4/IL-13 signals successfully controlled Th2-type chronic dermatitis. IL-4DM DNA treatment is a potent therapy for AD and related diseases.

Interleukin (IL)-4 plays a central role in Th2-cytokine-dominant inflammatory skin diseases such as atopic dermatitis (AD).^{1–3} IL-4 is responsible for the differentiation of allergen-specific Th2 cells together with its closely related cytokine IL-13 for the class switching of activated B cells to IgE-producing cells. The effects of IL-13 are similar to IL-4 on B cells, monocytes, and other cell types, but T cells appear to lack an IL-13 binding receptor component and do not respond to IL-13.⁴ The structural basis for the overlapping functions of IL-4 and IL-13 is a shared receptor subunit, and IL-4R α organizes intracellular signals in response to both cytokines.^{5,6} Signal transduction is induced by heterodimerization of the IL-4R α with a second subunit; which may vary according to the cell types. The specific inhibition of IL-4 can be achieved by antagonistic IL-4 mutants. Variants of human IL-4 that bind

to the receptor subunit IL-4R α , but not to the other subunit γ -chain (γ c) or IL-13R α 1 are competitive antagonists of IL-4.^{7,8} IL-13 is inhibited by similar variants, which form unproductive complexes with IL-4R α .^{5,9} The single-site human IL-4 mutant Y124D has been used as an IL-4/IL-13 inhibitor in various studies,^{7–17} but this variant retains some residual agonistic activity, which could be relevant for *in vivo* applications.^{7,8} In contrast, IL-4 and IL-13 double mutant R121D/Y124D lacks detectable activity and appears to be an effective antagonist for human IL-4 and IL-13.^{5,18}

We have recently developed a highly efficient murine IL-4 antagonist DNA (IL-4DM), in which the amino acids glutamine 116 and tyrosine 119 were changed for aspartic acid.¹⁹ This murine mutant DNA is analogous to the R121D/Y124D double mutant. IL-4DM binds with high affinity to the murine

IL-4R α without inducing signal transduction, and has no detectable activity upon the proliferation or differentiation of murine cells. An appropriate amount of IL-4DM completely inhibits responses by wild-type IL-4.¹⁹ Like its human analogue, the IL-4DM mutant is also an antagonist of IL-13 (B. Schnarr *et al.*, unpublished data³⁷). Recent experiments with monocytes from mice lacking a functional γ c gene showed that IL-4DM is a complete inhibitor of IL-4 in the absence of γ c as well.²⁰ In this study we have examined the effects of IL-4DM *in vivo*, using an AD model induced by the repeated exhibition of oxazolone (OX). The repeated application of a hapten such as OX on mice causes an initial delayed-type hypersensitivity that changes to an immediate-type response in the late phase with elevated IgE production and deviation of Th-cell responses. The skin lesions that appear in the late phase are compatible with the clinical findings as well as the cytokine profile observed in AD.^{21–23} The inhibitory effect of IL-4DM on IL-4 and IL-13 on the immune response was comparable with that of knockout mice lacking either IL-4²⁴ or IL-4R α . Treatment with IL-4DM prevented contact hypersensitivity responses with the increased production of interferon (IFN)- γ .

Materials and methods

Animals

BALB/c male mice aged 5 weeks were purchased from Japan SLC Co. (Shizuoka, Japan) and were used at the age of 6 weeks. Age-matched wild-type BALB/c mice were used as controls. All animals were cared for according to the ethical guidelines approved by the Institutional Animal Care and Use Committee of Mie University.

Reagents

The cDNA coding region of mouse IL-4 was amplified by a polymerase chain reaction (PCR) based on the cDNA sequence of mouse IL-4. The mouse IL-4 fragment was inserted into BamHI and EcoRI-filled in pcDNA3.1+ (Invitrogen, San Diego, CA, U.S.A.) under the TPA leader sequence, and then digested by BamHI and SacI. A QuickchangeTM Site-directed Mutagenesis kit (Stratagene, La Jolla, CA, U.S.A.) was used for the mutagenesis of mouse IL-4. The oligonucleotide primers used to prepare a mouse IL-4 double mutant (IL-4DM, Q116D/Y119D) were CTAAAGAGCATCATGGATATGGATGACTCGTAGTCTAGAG and CTCTAGACTACGAGTCATCCATATCCATGATGCTCTTTAG. The IL-4 mutant fragments were ligated into pcDNA3.1+.²⁵ Mouse IL-4, IL-4DM plasmid DNAs were purified using the Plasmid Mega kit (Qiagen, Chatsworth, CA, U.S.A.) and diluted with sterilized physiological saline. OX was purchased from Sigma (St Louis, MO, U.S.A.) and was dissolved in acetone/olive oil (4 : 1).

Administration of DNA

Mice were treated by intraperitoneal injection of 100 μ g of IL-4DM DNA on days 0, 7, 14, 21 and 28. A control plasmid

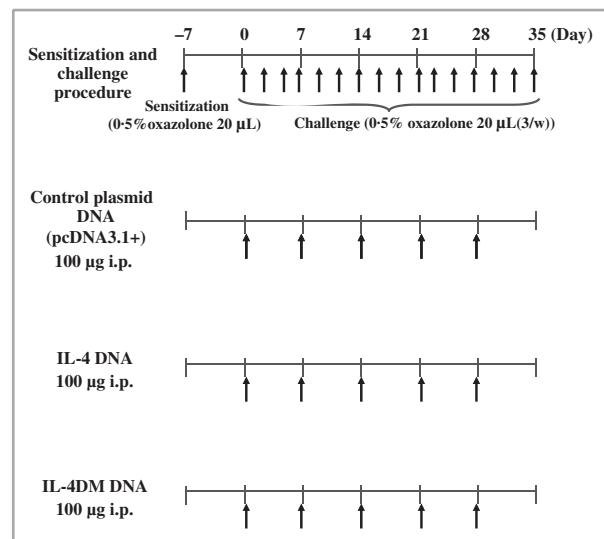


Fig 1. Schedule for induction of chronic contact hypersensitivity and administration of compounds. Mice received intraperitoneal (i.p.) injection of 100 μ g of each plasmid DNA on days 0, 7, 14, 21 and 28.

(pcDNA3.1+) vector and IL-4 DNA were also injected on the same day (Fig. 1).

Sensitization and challenge procedures

As shown in Figure 1, mice were initially sensitized by pasting 20 μ L of 0.5% OX solution to their left ear 7 days before the first challenge (day 7) and then 20 μ L of 0.5% OX solution was repeatedly applied on the left ear three times per week from day 0 as reported previously.²³ Ear swelling was measured with thickness gauge calipers before and 30 min after OX challenge to the pinna of the ear on day 35. The ear swelling response was expressed as the difference between the values taken before and 30 min after application.

Histological analysis

Ear skin specimens obtained 6 h after the final challenge on day 35 were fixed in 10% buffered neutral formaldehyde and embedded in paraffin wax. Histological sections were of 6 μ m thickness and they were stained with haematoxylin and eosin. The sections were also stained with 0.5% toluidine blue for the identification of mast cells. The cell counts were performed in six consecutive microscopic fields at $\times 400$ magnification.

Measurement of plasma IgE and plasma histamine

Blood was collected under ether anaesthesia 6 h after the last challenge. Plasma IgE levels were determined by a sandwich enzyme-linked immunosorbent assay (ELISA). In brief, 96-well immunoplates (Corning Inc., Corning, NY, U.S.A.) were coated with 100 μ L of an antimouse IgE capture antibody (2 μ g mL⁻¹) (BD PharMingen, San Diego, CA, U.S.A.) overnight at 4 $^{\circ}$ C. Plasma samples of 100 μ L were diluted 60-fold with PBS

containing 10% fetal calf serum (FCS) were placed in the wells. After incubation for 1 h at room temperature, 100 µL of biotin-conjugated antimouse IgE antibody ($2 \mu\text{g mL}^{-1}$ in blocking buffer) (BD PharMingen) was added to each well. The plates were incubated at room temperature for 1 h, followed by six washes, incubated with 100 µL of horseradish peroxidase avidin D (FUNAKOSHI, Tokyo, Japan) 1 : 1000 in blocking buffer, and then incubated for 30 min at room temperature. A substrate solution of 100 µL containing 1.5 mg ABTS (Sigma-Aldrich, St Louis, MO, U.S.A.) in 5 mL of a 0.1 mol L⁻¹ citric acid solution was added, and kept for 30 min at room temperature in a dark place. Thereafter the reaction was terminated by adding 50 µL of 2 mol L⁻¹ H₂SO₄, and the optical density of each well at 405 nm was determined by using a microplate reader. A standard curve was prepared using mouse anti-TNP IgE standard (BD PharMingen). Plasma histamine levels were analysed using the commercial sandwich ELISA kit from Immunoteck (Marseille, France) according to the manufacturer's protocol.

Purification of mRNA from mouse ears

At 6 h after the final challenge, the skin of the left ear was sampled. The specimen was homogenized and the total RNA was extracted using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instruction; 1 mL of homogenate was vigorously mixed with 200 µL of chloroform, and centrifuged at 12 000 g for 15 min at 4 °C. The aqueous phase was separated and mixed with 0.5 mL of 2-propanol (Nacalai Tesque, Kyoto, Japan) to precipitate RNA. After centrifugation, the precipitate was washed with 1 mL of 75% ethanol (Nacalai Tesque) and dried. RNA was suspended in 50 µL of RNase-free water, and the concentration was measured based on the absorbance at 260 nm, and the quality was confirmed by electrophoresis. cDNA was prepared from 10 µg of mRNA using archive kit (ABI, Foster City, CA, U.S.A.) according to the manufacturer's protocol.

Cytokine mRNA expression in skin

The transcriptional activity in the lesional skin samples was measured with a PCR. The amplification of cDNA was performed in 50 µL of a master mixture containing 0.5 µg of cDNA, 200 nmol deoxynucleotide triphosphate, 5 µL of PCR buffer, 2 U of Taq polymerase (ABI) and 2 µmol of each specific primer for the DNA of interest. The following primers were used for PCR reactions (5'-3'), mouse IFN- γ : TCAAGTGGCATAGATGTGGAAGAA and TGGCTCTGCAGGATTTTCATG; mouse IL-2: CCTGAGCAGGATGGAGAATAACA and TCCAGAACATGCCGAGAG; mouse IL-4: CACTGACGGCACAGAGCTATTGATG and TCATGGTGCAGCTTTCGATGAATC; mouse IL-10: CTCTTACTGACTGGCATGAGGATCAGCAGG and TCTTCACCTGCTCCACTGCTTGCTCTTAT; mouse IL-12: TCCTGCACTGCTGAAGACATC and TCTCGCCATTATAGATTTCAGAGAC; mouse IL-13: AGACCA-GACTCCCCTGTGCA and TGGGTCTGTAGATGGCATTG; mouse β -actin: TGGAACTCTGTGGCATCCATGAAAC and TAAAACG-CAGCTCAGTAACAGTCCG.²⁶ PCR was performed under the

following conditions: 95 °C for 5 min, followed by 35 or 40 cycles of 95 °C for 30 s, 56 °C (IFN- γ , IL-12) or 60 °C (IL-2, IL-4, IL-10, IL-13, β -actin) for 30 s, and 72 °C for 1 min were carried out. After the final cycle, the temperature was maintained at 72 °C for 7 min. PCR amplified fragments were electrophoresed through 1.5% agarose gels in tris-acetate EDTA buffer containing ethidium bromide, and the gels were scanned under ultraviolet light. The mRNA of β -actin was used as an internal control. The signal intensity of each reverse transcriptase (RT)-PCR product was estimated using an ATTO Lane & Spot Analyzer (ATTO, Shizuoka, Japan).

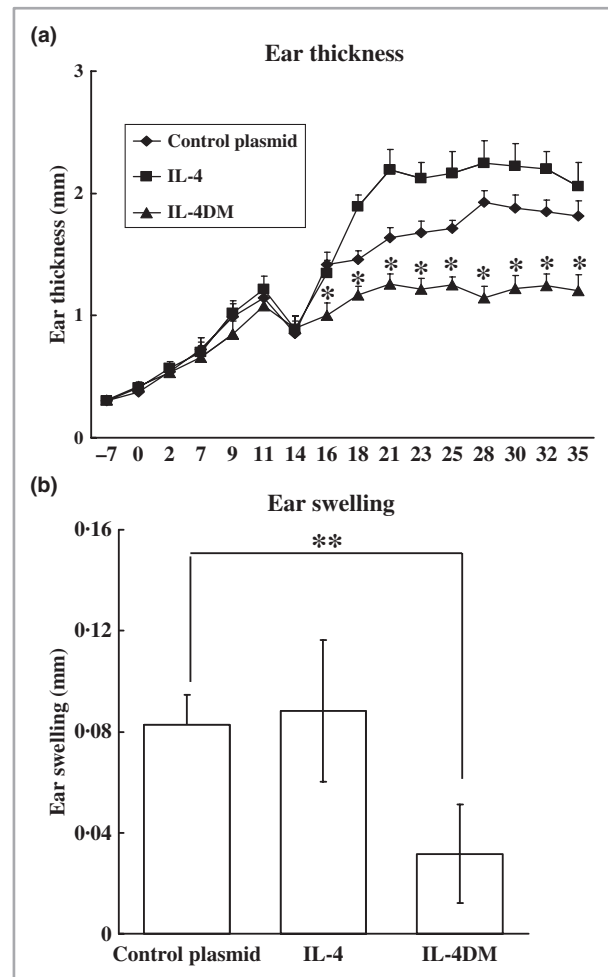


Fig 2. The effects of interleukin (IL)-4DM, IL-4, and control plasmid (pcDNA3.1) on ear swelling induced by repeated application of oxazolone (OX). (a) Ear thickness was measured before each OX challenge. Each point represents the mean $\pm$ SD of seven or eight mice. * $P < 0.05$: significantly different from the control group and IL-4 (Student's *t*-test). (b) Inhibition of the effector phase of chronic hypersensitivity by IL-4DM, IL-4, and control plasmid DNA transfer. The ear swelling was measured 30 min after applying OX. The ear swelling in the IL-4DM groups was significantly suppressed compared with those in the IL-4 and control plasmid DNA groups. *Significant difference from the control by Student's *t*-test at $P < 0.05$.

Cytokine production from splenocytes

A suspension of 2×10^6 splenocytes were made in a solution of 200 μ L RPMI-1640 medium (Nikken Bio Medical Laboratory, Kyoto, Japan) containing 10% fetal bovine serum (FBS; Biowest, Nuaillé, France), 50 UI penicillin, 50 μ g mL⁻¹ streptomycin, and 5 μ g mL⁻¹ soluble antimouse CD3 (BD Bioscience), and 10 μ g mL⁻¹ antimouse CD28 (BD Bioscience). Cells were dispensed in triplicate into 96-well flat-bottomed microplates (Sumitomo Bakelite, Tokyo, Japan). After incubation for 48 h at 37 °C in a humidified incubator (5% CO₂), culture supernatants were collected and analysed for IFN- γ (Quantikine; R&D Systems, Minneapolis, MN, U.S.A.) or IL-4 (Quantikine; R&D Systems) production with an ELISA according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using Student's *t*-test and Mann-Whitney *U*-test. Values are expressed as mean $\pm$ SEM. A 95% confidence limit was taken as significant ($P < 0.05$).

Result

Ear thickness with the treatment of IL-4DM, IL-4, or control plasmid

In the control group, the ear thickness increased from the beginning of the challenge, and increased gradually through the experiments (Fig. 2a). The agonistic IL-4 DNA treatment augmented increase of the ear thickness after day 16. In contrast, IL-4DM DNA treatment significantly suppressed increase of the ear thickness compared with that of control plasmid or IL-4DNA-treated mice.

Effects of IL-4DM on the oxazolone-induced acute-phase ear swelling

The ear swelling was also measured 30 min after OX application on day 35, and the difference between before and 30 min after application was calculated. IL-4DM DNA treatment suppressed the ear swelling significantly compared with that of the control DNA-injected group (Fig. 2b). However, IL-4DNA showed no suppressive effects.

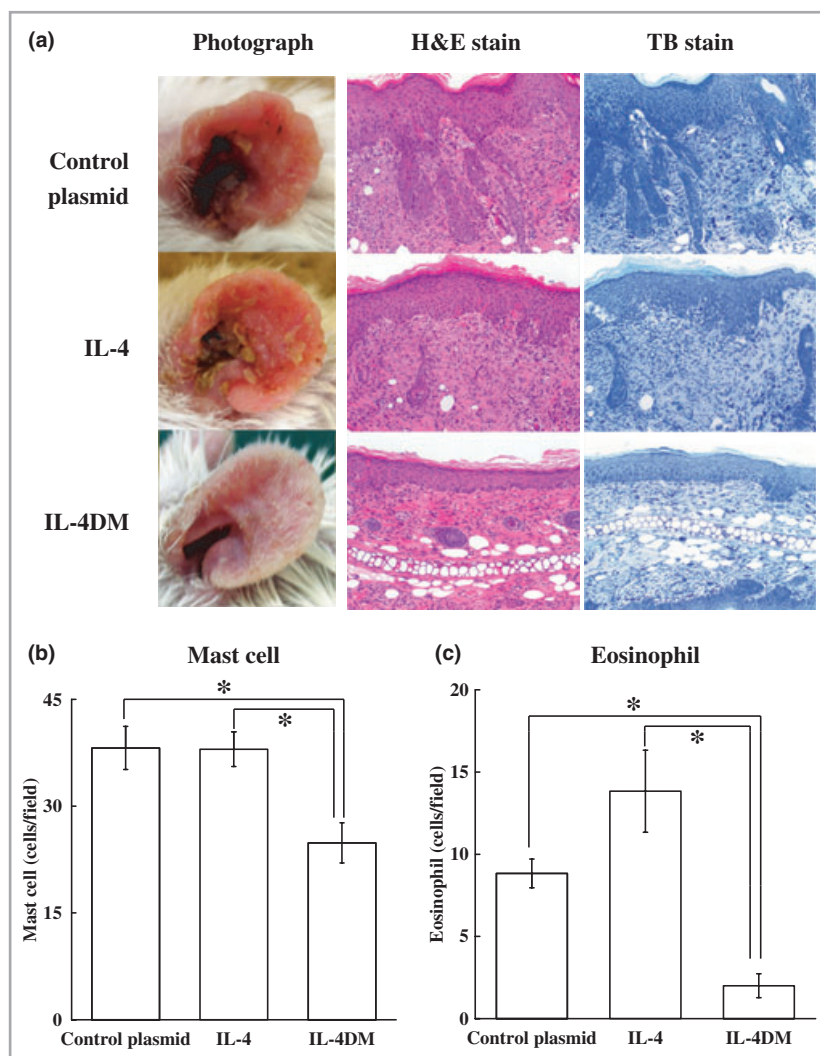


Fig 3. (a) Representative photographs and histological feature of oxazolone (OX)-treated skin lesion. OX-sensitized ear revealed hyperkeratosis, acanthosis, and parakeratosis in control and interleukin (IL)-4-treated mice. An increased number of infiltrating lymphocytes, macrophages and mast cells was observed in the skin lesions, all of which are typical histological findings observed in patients with atopic dermatitis. In contrast, acanthosis was clearly suppressed, and skin infiltration of granulocytes, eosinophils, and mast cells was decreased in the IL-4DM-treated mice as compared with control plasmid-treated mice (original magnification $\times 200$). (b) The number of dermal mast cells was counted, and found to be decreased in the IL-4DM-treated mice. (c) The number of dermal eosinophils was also counted in 10 high power fields. The skin infiltration of eosinophils was significantly decreased in the IL-4DM-treated mice. Data are expressed as the mean $\pm$ SEM. *Significant difference by Student's *t*-test at $P < 0.05$.

Histological findings and mast cell counts in the inflamed skin

In control plasmid-treated mice and IL-4 DNA-treated mice, severe dermatitis was observed on the earlobe. A drastic decrease of inflammation was observed in IL-4DM DNA-treated mice (Fig. 3a). Histological examination on the OX-challenged ear skin revealed hyperkeratosis, acanthosis and parakeratosis in both of the control and IL-4-treated mice. An increased number of infiltrating lymphocytes, macrophages and mast cells was observed in the skin lesions in control DNA and IL-4 DNA-treated mice. These findings are comparable with those of AD skin lesions. In contrast, the acanthotic changes and infiltration of granulocytes, mast cells, and eosinophils were significantly suppressed in the IL-4DM DNA-treated mice compared with those of control DNA- or IL-4 DNA-treated mice (Fig. 3b,c). Interestingly, IL-4 DNA treatment increased eosinophil counts compared with control DNA treatment.

Plasma IgE and histamine levels

The total plasma IgE level was increased by repeated OX challenges (Fig. 4a). IL-4 DNA treatment showed no agonistic effects in the plasma IgE level; however, IL-4DM DNA treatment significantly suppressed the levels of plasma IgE. The plasma histamine level was also significantly increased in the control DNA- or IL-4 DNA-treated mice; however, IL-4DM DNA treatment significantly suppressed the plasma histamine levels (Fig. 4b).

Cytokine mRNA expression levels

To determine the effects of IL-4DM on cytokine production in the inflamed skin lesions, mRNA expression of Th1 and Th2 cytokines was analysed. The IFN- γ mRNA expression was significantly increased in IL-4DM DNA-treated mice ear compared with that of control DNA-treated samples (standardized by β -actin expression).

However, no remarkable difference in other cytokine mRNA expression was observed among three different DNA-treated samples (Fig. 5a,b).

Concentration of IFN- γ and IL-4 in splenocyte cell culture supernatants

To know the effects of IL-4DM DNA therapy in the systemic immune system, the concentration of IFN- γ in splenocyte cell culture supernatants was measured by ELISA. The IFN- γ level in the IL-4DM-treated samples was significantly higher than that in the control DNA- or IL-4 DNA-treated samples (Fig. 6a). We also measured the concentration of IL-4 in splenocyte cell culture supernatants by ELISA for mouse IL-4. The IL-4 level in the IL-4DM-treated samples was as high as the IL-4 DNA-treated samples. These were higher than that of the control DNA-treated samples (Fig. 6b).

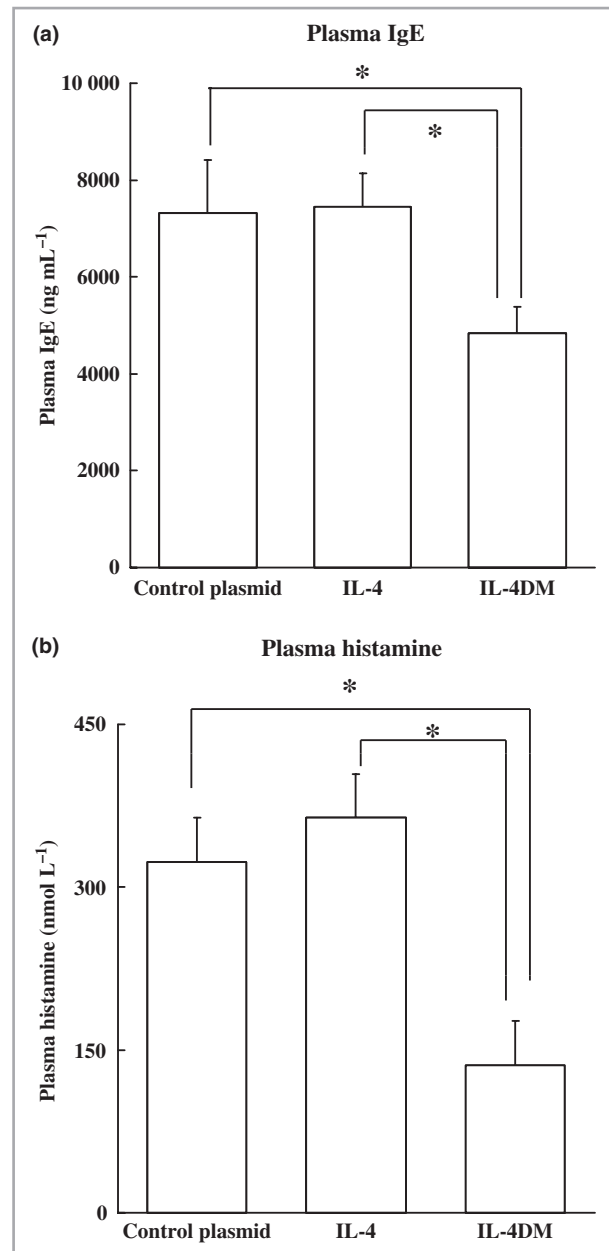


Fig 4. Plasma IgE and histamine levels. (a) Plasma IgE level was decreased in interleukin (IL)-4DM treated mice. (b) Inhibition of the production of plasma histamine was observed in IL-4DM DNA-treated mice. *Significant difference from the IL-4 and control by Mann-Whitney U-test at $P < 0.05$.

Discussion

Several previous studies have shown that AD is a chronic dermatitis with a predominance of Th2 cytokines in the lesional skin,²⁷⁻²⁹ and that Th2 cytokines play a critical role in the pathogenesis of dermatitis.²⁸ IL-4 is one of the Th2 cytokines that affects the function of different cell types including T cells, B cells, mast cells, monocytes/macrophages, endothelial cells, fibroblasts, dendritic cells, Langerhans cells

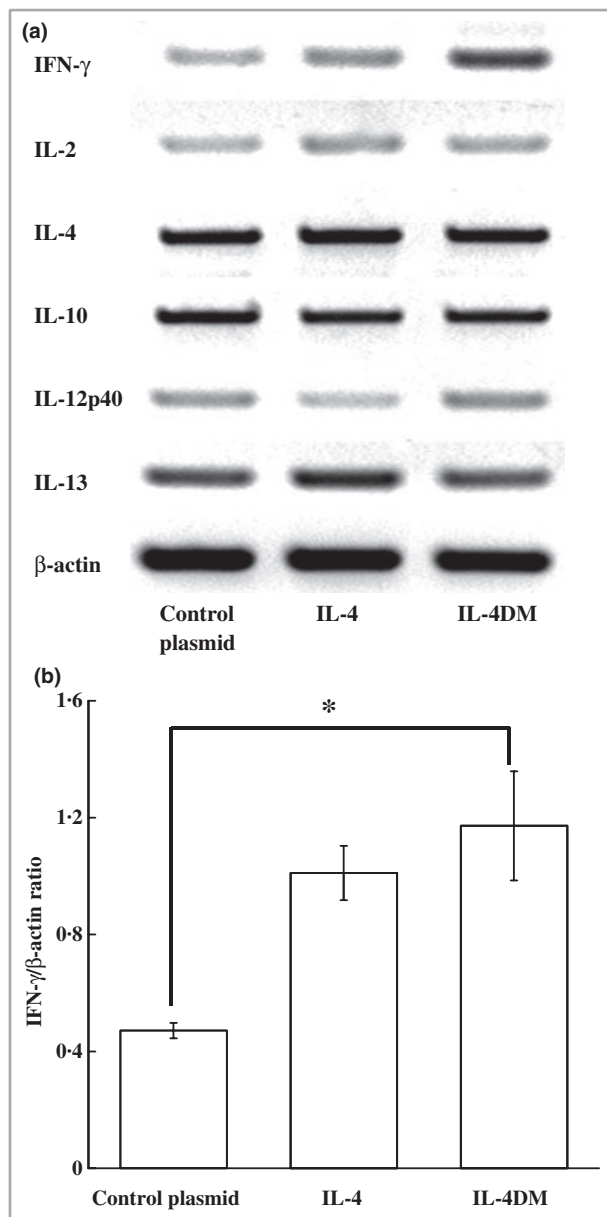


Fig 5. (a) Reverse-transcriptase polymerase chain reaction analysis of cytokine mRNA expression 6 h after oxazolone (OX)-sensitization. The cDNAs were amplified for respective cycles of six cytokines and β -actin, subjected to electrophoresis, and visualized with ethidium bromide. Representative results under optimal conditions are shown. Although almost all Th1 and Th2 cytokine levels were unchanged, mRNA expression for interferon (IFN)- γ was increased in IL-4DM-treated mice. (b) The level of mRNA expression of IFN- γ was expressed as the value relative to that for β -actin. The IFN- γ level in the IL-4DM group is significantly higher than that of control plasmid groups. *Significant difference from the control by Student's *t*-test at $P < 0.05$.

and keratinocytes. Because of this broad-spectrum action, IL-4 is believed to play a crucial role in the pathogenesis of AD.^{30,31} In the present study, we employed a contact hypersensitivity model by the repeated application of OX, which mimics the histological phenotype of AD in humans; this

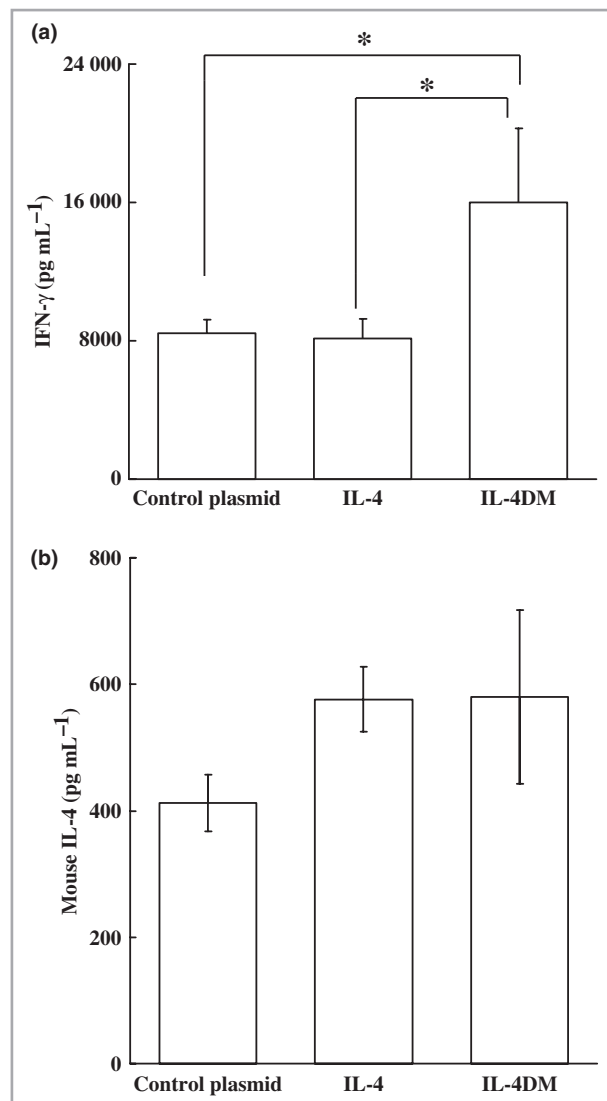


Fig 6. Cytokine production from splenocytes in chronic hypersensitivity mice. Interferon (IFN)- γ and interleukin (IL)-4 production from splenocytes was measured. (a) Actual IFN- γ protein production was increased in the IL-4DM-treated mice. (b) IL-4 levels did not reach the significance, but showed a tendency to increase in the IL-4 and IL-4DM mice. *Significant difference from the control by Mann-Whitney U-test at $P < 0.05$.

model also showed increased levels of Th2 cytokines in the lesional skin as reported by Kitagaki *et al.*²¹

Immunotherapy such as the direct blocking of Th2 responses with neutralizing antibody against Th2 cytokines, the soluble form of IL-4 receptor (IL-4R), or antagonistic IL-4 mutant proteins have been used for the treatment of asthma.³²⁻³⁴ These proteins directly inhibit IL-4 binding thereby inhibiting host immune responses. A previous study by Nishikubo *et al.*²⁵ showed inhibition of immune responses by using IL-4 mutant protein for at least 50 weeks. However, results from these experimental animals have shown that the application of these trials to humans is difficult. Because the pharmacokinetic half-life of IL-4 mutant and sIL-4R protein

are very short *in vivo* (IL-4 mutant: $t_{1/2} = 0.83$ h; sIL-4R: $t_{1/2} = 4.6$ h),^{35,36} huge amounts of these molecules are required in plasma to maintain a long period of inhibitory action on allergic inflammation. In fact, administration of these molecules was required many times in high doses from the sensitization to the challenge periods.^{35–37} In the present study, we demonstrated a remarkable antagonistic effect of IL-4 mutant DNA applied in a form of vaccination, as a potent new type of immunogene therapy for AD. In previous studies in which gene therapy and DNA vaccines were used in combination with a cytokine gene for tumours or pathogens, effective immune responses to antigen were recognized even in the absence of detectable plasma levels of cytokines. Recently, we also reported that administration of plasmid DNA coding IL-4 cDNA completely inhibited the development of insulinitis, which is one of the Th1-type autoimmune diseases, although no IL-4 was detected in plasma.³⁸ These results suggest that genes applied as a DNA vaccine express and supply products to the host continuously. To occupy the IL-4/IL-13 receptors, a continuous supply of IL-4DM is needed but not bolus application. Therefore, IL-4DM applied as a DNA vaccine might inhibit the allergic inflammation by persistent secretion of mutant IL-4 over a long period in a limited amount.

As we had expected, IL-4DM mitigated phenotypal and histological changes such as severe oedema, inflammatory cell infiltration and epidermal hyperplasia. IL-4DM also significantly decreased the number of dermal mast cells. IL-4 is known to be a potent activator of mast cells. Mast cells, which participate in the inflammatory cascade, serve as an abundant source of Th2 cytokines as well as inflammatory mediators.^{39–41} Therefore, inhibition of mast cell activation is another possible mechanism through which IL-4DM ameliorates inflammatory responses in the present model of dermatitis. Eosinophil infiltration into the dermis has been well documented in AD.⁴² In this study, an increased number of eosinophils was observed in contact hypersensitivity skin lesions, and was dramatically inhibited by IL-4DM treatment. Inhibition of cellular infiltration in IL-4DM mice may be due to suppression of IL-4-mediated immunological events such as a decreased expression of cellular adhesion molecules on endothelial cells.⁴³

Injected IL-4DM and IL-4 DNA are trapped by monocytes/macrophages by phagocytosis. They may migrate to lymph nodes or spleen and show systemic effects. In fact, we could observe a high concentration of IL-4 in cultured splenocytes from IL-4DM DNA injected mice by ELISA. Unfortunately, there is no specific anti-IL-4DM antibody or anti-IL-4DM ELISA. The standard ELISA used in this study could not differentiate the natural mouse IL-4 and mutant IL-4 protein; these findings are consistent with the previous report.²⁵ The plasma IL-4 levels in the agonistic IL-4DNA-treated mice were consistent with those of the IL-4DM DNA-treated mice. Therefore, we speculate that exogenously applied IL-4DM DNAs were expressed the same as IL-4 DNAs, and showed systemic immunological effects.

IFN- γ production increased systemically and locally in mice treated with IL-4DM DNA. Repeated OX treatments cause expansion both of Th1 and Th2 cells. IL-4DM DNA therapy interfered with the development of the Th2 milieu. Subsequently, IFN- γ production and mRNA expression might become abundant locally and systemically.

Tissue-specific gene transfer could be achieved naturally and effectively through the cell specificity of virus receptors.⁴⁴ However, there may be a risk of vector toxicity through viral infection of host cells. Also, the limited size of transgenes is often a serious obstacle. Moreover, immune responses to viral vectors are also induced, and the effects of transgenes are eliminated by immune responses to the vectors. For human applications, the efficacy and safety of any delivery system for gene transfer are always of major concern. Nonviral approaches are advantageous in immunogene therapy. DNA vaccines are capable of inducing potent biological effects in a variety of experimental systems.⁴⁵ One of the characteristic features of DNA vaccines is their ability to induce long-lasting immunity. The animals that had been treated with IL-4DM DNA did not develop severe allergic inflammation even before or after antigen sensitization.

In the present study, we showed the beneficial effects of immunogene therapy with IL-4 mutant DNA in an experimental model for AD. An IL-4 mutant DNA vaccination is a potent new tool for the systemic treatment of AD.

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