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Comparison of the presentation of atopic dermatitis induced by trinitrochlorobenzene and house dust mite in NC/Nga mice

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ABSTRACT

Background: Atopic dermatitis (AD) is a common chronic inflammatory skin disease. To understand AD, there have been many trials establishing AD animal models. Although various trials to establish AD animal models have been existed, even the mechanisms of AD in animal models are not enough clarified.
Objectives: This study assessed AD characteristics induced in Nishiki-nezumi Cinnamon/Nagoya (Nc/Nga) mice following trinitrochlorobenzene (TNCB) treatment for different periods and house dust mite (HDM) treatment to compare each model's immunological patterns, especially with cytokine antibody array tool.
Methods: In this study, we exposed Nc/Nga mice to TNCB or HDM extract to induce AD. Nc/Nga mice were divided into 4 groups: control, TNCB 2 weeks-treated, TNCB 8 weeks-treated, and HDM-treated groups. After AD induction, all mice were evaluated by serum immunoglobulin E (IgE) concentration and serum cytokine antibody assays, scoring of skin lesions, scoring of scratching frequency, and histological analysis.
Results: The results showed significant differences between groups in serum IgE concentration, skin lesion scores, and scratching frequency. The analysis results for serum cytokine antibody arrays showed that in the TNCB 8 weeks- and HDM-treated groups, but not in the TNCB 2 weeks-treated group, expressions of genes related to the immune response were enriched. Among the histological results, the skin lesions in the HDM-treated group were most similar to those of AD.
Conclusions: We confirmed that immunological pattern of AD mice was markedly different between HDM and TNCB treated groups. In addition, the immunological pattern was quietly different dependent on TNCB treated duration.

Keywords: Atopic dermatitis; Nc/Nga mouse; trinitrochlorobenzene; house dust mite; cytokines

INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory skin disease accompanied by clinical signs such as severe pruritus, dryness, erythema, edema, excoriation, or

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contribution

Conceptualization: Chung JY; Data curation: Kang MS, Ahn JO; Formal analysis: Chung JY, Choi JH; Funding acquisition: Chung JY; Investigation: Kim YH, Kim TH; Methodology: Kang MS, Ahn JO; Project administration: Chung JY; Supervision: Chung JY, Choi JH; Validation: Chung JY, Choi JH; Visualization: Kang MS, Ahn JO; Writing - original draft: Kim YH, Kim TH; Writing -review & editing: Chung JY, Choi JH.

lichenification. In human medicine, up to 20% of people suffer from AD [1]. Not only in human medicine but also in veterinary medicine, AD is a common dermatosis defined as a genetically predisposed inflammatory and pruritic skin disease [2].

The pathogenesis of AD can be summarized as follows [3-5]: allergens invade through a defective skin barrier and are then recognized by antigen-presenting cells, Langerhans cells, or dermal dendritic cells in the epithelium. In the acute phase, this is followed by the differentiation of T helper (Th) 0 cells into Th2 cells, while in the chronic phase Th0 cells differentiate into Th1 cells, accompanied by the release of various cytokines. These processes induce B cells to produce IgE, which, in turn, induce mast cells and eosinophils to stimulate degranulation, releasing several inflammatory mediators such as histamine, bradykinin, prostaglandin, and leukotriene. Meanwhile, Th2 cells migrate to the skin and release various cytokines to induce pruritus [6]. Scratching, a consequence of pruritus, induces mechanical injury and release of cytokines to disrupt the skin barrier and attract eosinophils to the skin layer. Many inflammatory cells can infiltrate skin tissue, and inflammatory cytokines released from inflammatory cells stimulate Th1 cell differentiation and activate cellular immunity [4,7].

During previous attempts to further the understanding of AD, there have been many studies into establishing AD animal models [8-10]. Those AD animal models can be categorized into 3 groups: 1) models induced by sensitizers; 2) transgenic mice which over-express or lack the expression of molecules; 3) mice that spontaneously develop AD-like skin lesions [11]. Nishiki-nezumi Cinnamon/Nagoya (Nc/Nga) mice are included in the latter group of mice that spontaneously develop AD-like skin lesions. Nc/Nga mice are members of a mutation strain developed at Nagoya University in Japan in 1997 and are the first AD mouse model to be reported [12]. The skin changes of Nc/Nga mice develop spontaneously under conventional conditions, not under specific-pathogen-free conditions, and the changes closely mimic human AD [13]. Some studies have suggested that exposure to a conventional environment is not sufficient to produce AD in NC/Nga mice; however, this can be resolved by applying a sensitizer to the surface of the skin, thus easily leading to AD [14]. Trinitrochlorobenzene (TNCB) and house dust mite (HDM) allergen are representative sensitizers. TNCB is a hapten that is commonly used to induce AD and has been thought to evoke a primarily Th1-dominated response [11]. The HDM allergen is very common in real life. Clinical study had provided evidence that HDM allergen is associated with AD [9]. *Dermatophagoides farinae* is a representative HDM that can produce symptoms similar to those of AD. The HDM allergen-related pathogenesis is unclear but it is reported that application of a HDM extract to the skin can increase the expressions of Th1/Th2/Th17 related cytokines [15].

Currently, there are few studies comparing the various AD animal models. In this study, we assessed AD characteristics induced in Nc/Nga mice following TNCB treatment for different periods (short period, 2 weeks and long period, 8 weeks) and HDM treatment to compare each model's immunological patterns.

MATERIALS AND METHODS

Animals

Eight-week-old female NC/Nga mice were purchased from Central Laboratory Animal Inc. (Korea). The mice were housed in an air-conditioned room maintained at 24°C ± 2°C and 55% ± 15% humidity. Protocols for care and use of animals in this study were in compliance with

guidelines and were approved by the Kangwon National University Institutional Care and Animal Use Committee (KW-180705-4).

Twenty NC/Nga mice (8-week-old females) were used during this study. Mice were assigned to one of 4 groups; control group, TNCB 2 weeks-treated group, TNCB 8 weeks-treated group, and the HDM-treated group (n = 5 in each group).

Induction of AD in the NC/Nga mice

TNCB and HDM were used to induce AD [12,16]. For the control group, the hair on the back of the NC/Nga mice was shaved using an electric shaver, followed by treatment with saline twice a week for 4 weeks. For the TNCB 2 weeks-treated group, the hair on the back of the NC/Nga mice was shaved using an electric shaver, followed by treatment with 100 μL of 2% TNCB (Sigma-Aldrich, USA) 3 times a week for 2 weeks. For the TNCB 8 weeks-treated group, the hair on the back of the NC/Nga mice was shaved using an electric shaver, followed by treatment with 100 μL of 2% TNCB 3 times a week for 2 weeks. After then 100 μL 0.2% TNCB was applied 3 times a week for 6 weeks in the TNCB 8 weeks-treated group. TNCB was prepared by dissolving TNCB in a 4:1 mixture of acetone and olive oil.

For the HDM-treated group, the hair on the back of the NC/Nga mice was shaved using an electric shaver, followed by treatment with 100 μL of 4% (w/v) sodium dodecyl sulfate (SDS; Sigma-Aldrich, USA) to disrupt the skin barrier. After drying with 4% SDS, 100 mg/mouse of HDM allergen (HDM; Biostir Inc., Japan) was applied to the prepared skin area, with the HDM application repeated twice per week for 4 weeks (**Fig. 1**).

Serum IgE concentration assay

Serum was collected from sacrificed mice, and the concentration of total IgE in the serum was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Fujifilm Wako Shibayagi Corporation, Japan). All of the ELISA procedures were performed following the manufacturer's instructions. Upon completion of the assay procedure, the plate was read at 450 nm using a SpectraMax ABS Plus Microplate Reader (Molecular Devices, LLC, USA). All of the ELISA analysis was replicated.

Serum cytokine antibody array

Serum from 5 sacrificed mice in each group was pooled into one sample and 100 μL of pooled serum was used for the array protocol. The concentration of sample was measured with BCA

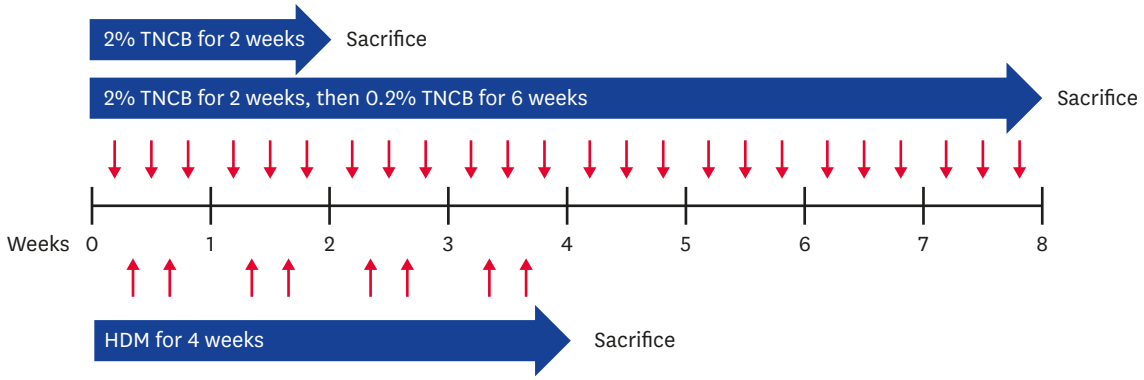

Fig. 1. Scheme of AD induction in NC/Nga mice.
AD, atopic dermatitis; NC/Nga, Nishiki-nezumi Cinnamon/Nagoya; TNCB, trinitrochlorobenzene.

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protein assay kit (Pierce,USA) using Multi-Skan FC (Thermo, USA). And the purity of sample was confirmed on ultraviolet (UV) spectrum. The pooled serum was diluted 1:10 and probed to determine the cytokine profile according to the manufacturer's instructions (RayBiotech, Inc., USA).

The slide scanning was performed using GenePix 4100A Scanner (Axon Instrument, USA). The slides were scanned at 10 μm resolution, optimal laser power and photomultiplier tube. After got the scan image, they were quantified with GenePix Software (Axon Instrument). After analyzing, the data about protein information was annotated using UniProt DB. Relative fold changes were calculated by dividing the value obtained from the treated groups by that from the control group.

Scoring of skin lesions

The extents of 1) erythema/hemorrhage, 2) scarring/dryness, 3) edema, and 4) excoriation/erosion were individually scored as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The total skin score was the sum of the individual scores [17]. Scoring was performed every week during the experiment period. All of the scoring was fulfilled by 2 different observers repeatedly for the exclusion of bias.

Scoring of scratching frequency

The frequency of scratching on facial or dorsal skin was determined based on counting the number of scratches in a 5-min period. The methodology used for behavioral observations was a modification of the methodology of Kobayashi et al. [18]. Scratch counting was performed every week during the experiment period. All of the scoring was fulfilled by 2 different observers repeatedly for the exclusion of bias.

Histological analysis

For histological analysis, mice were anesthetized with a high dose of Zoletil 50 (Virbac, France) on the last day of the experiment and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by fixation with 4% paraformaldehyde in 0.1 M PBS. Subsequently, dorsal skin tissues were removed and post-fixed for 24 h in the same fixative at 4°C. The fixed tissues were dehydrated with a graded series of alcohol concentrations before being embedded in paraffin. Paraffin-embedded tissues were sectioned using a microtome (Leica Microsystems GmbH, Germany) into 5 μm sections and then mounted onto silane-coated slides (Muto Pure Chemicals Co., Ltd, Japan). The sections were stained with hematoxylin and eosin (H&E) and toluidine blue (TB) staining according to a standard protocol. The cell density was expressed as the number of cells per 250 mm^2 for each section. All of the histological analysis was replicated.

Statistical analysis

Statistical analyses of all data were performed using the GraphPad Prism (ver. 5.01; GraphPad, USA) statistical analysis software. The values shown represent the means of experiments performed for each experimental group. Differences among means were identified by performing Mann-Whitney and Kruskal-Wallis tests. A $p < 0.05$ was considered to indicate significance.

RESULTS

Comparison of serum IgE concentration between groups

The serum IgE concentrations among the groups were significantly different ($p = 0.001$). The serum IgE concentrations in the TNCB 2 weeks-treated group, TNCB 8 weeks-treated group, and the HDM-treated group were significantly different than that of the control group ($p = 0.008$). Moreover, the serum IgE concentration in the TNCB 2 weeks-treated ($p = 0.008$) and TNCB 8 weeks-treated ($p = 0.016$) groups were significantly different than that of the HDM-treated group. However, a comparison of the serum IgE concentrations of the TNCB 2 weeks-treated and TNCB 8 weeks-treated groups revealed no significant difference ($p = 0.222$) (**Fig. 2**).

Comparison of serum cytokine antibody arrays between groups

Antibody array scatter plots are presented in **Fig. 3A**. The plots illustrate changes in signal intensities between the control group and the TNCB 2 weeks-treated group, between the control group and the TNCB 8 weeks-treated group, and between the control group and the HDM-treated group, with red and green lines indicating 2-fold up- or down-regulated intensities, respectively (**Fig. 3A**). The antibody array analysis identified 53 significantly up-regulated proteins in the comparison between the control and TNCB 8 weeks-treated groups (> 2-fold changes in the normalized value; *t*-test *p*-value < 0.05; **Table 1**). Between the control and HDM-treated groups, there were 5 significantly up-regulated and 3 significantly down-regulated proteins (> 2-fold changes in the normalized value; *t*-test *p* value < 0.05; **Table 2**). However, there were no significantly (fold changes > 2) up- or down-regulated proteins in the TNCB 2 weeks-treated group when compared to the control group.

All genes identified from the antibody array analysis were further analyzed according to categories within The Database for Annotation, Visualization and Integrated Discovery and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Interestingly, 68 components, as determined by applying the Gene Ontology_Biological Process (GO_BP), in the list of proteins that were regulated by TNCB for 8 weeks were significantly enriched. Among them, the top 10 enriched GO_BP terms were immune response, response to lipopolysaccharide, inflammatory response, chemotaxis, positive regulation of inflammatory response, wound healing, positive regulation of ERK1 and ERK2 cascades, positive regulation of MAPK

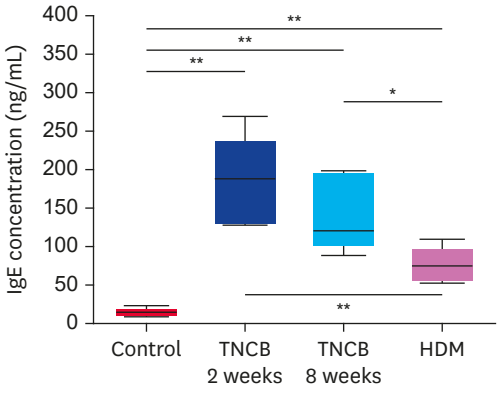

Fig. 2. Comparison of serum IgE concentrations between groups. The serum IgE concentrations were significantly different among the groups with the exception of the serum IgE concentrations of the TNCB 2 weeks-treated and TNCB 8 weeks-treated groups.
IgE, immunoglobulin E; TNCB, trinitrochlorobenzene; HDM, house dust mite.
$^{*}p < 0.05$; $^{**}p < 0.01$.

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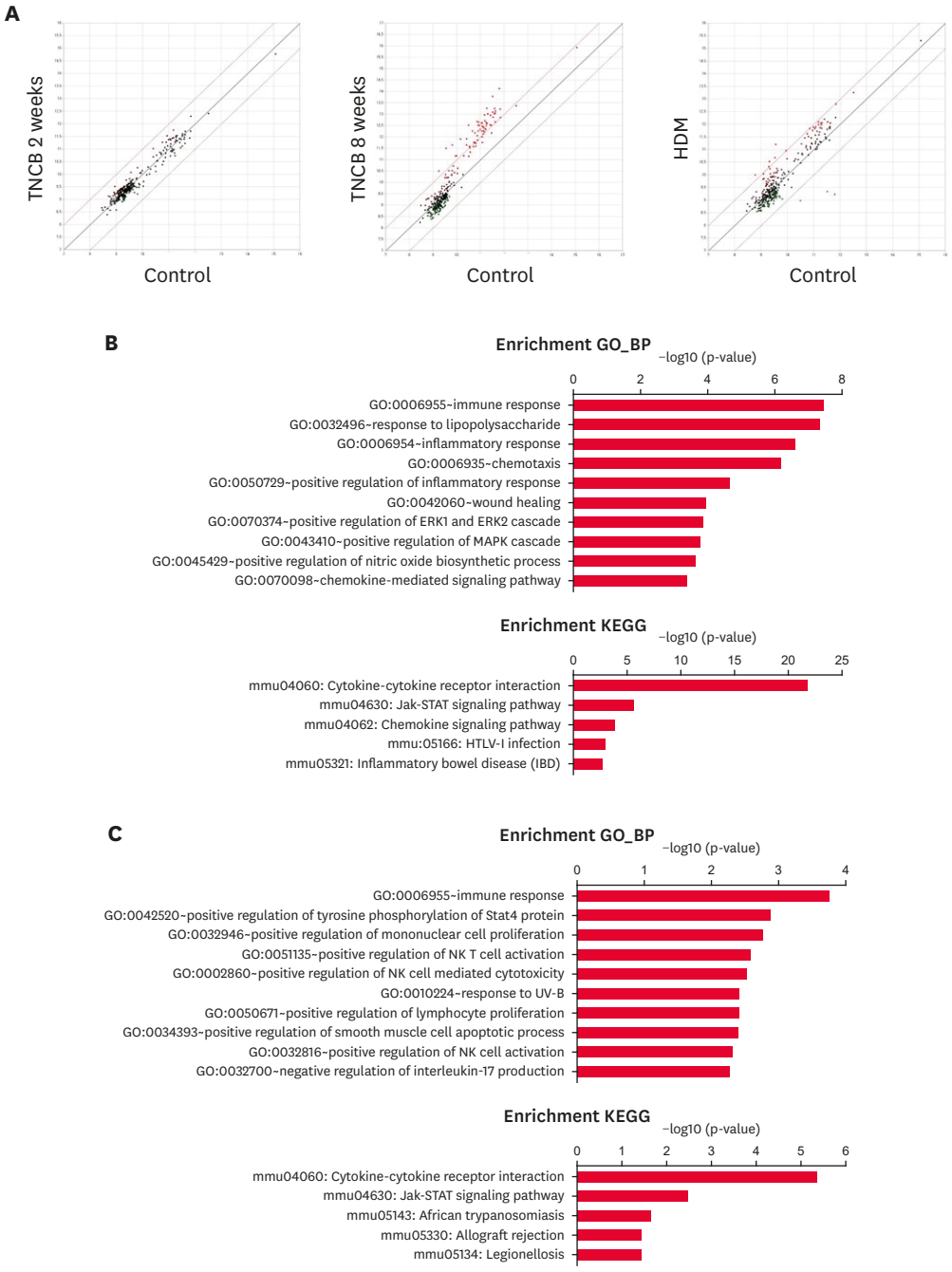

Fig. 3. (A) Changes in signal intensities between control and TNCB 2 weeks-treated groups, between control and TNCB 8 weeks-treated groups and between control and HDM-treated groups. (B) Functional analysis of antibody array results following TNCB treatment for 8 weeks. (C) Functional analysis of antibody array results following HDM treatment.
TNCB, trinitrochlorobenzene; HDM, house dust mite; GO_BP, Gene Ontology_Biological Process; KEGG, Kyoto Encyclopedia of Genes and Genomes.

cascade, positive regulation of nitric oxide biosynthetic process, and chemokine-mediated signaling pathway (**Fig. 3B**). Among KEGG categories, there were 14 pathways that were significantly enriched in the TNCB 8 weeks-treated group. Among them, cytokine-cytokine receptor interaction, Janus kinase-signal transducers and activators of transcription (Jak-STAT) signaling pathway, chemokine signaling pathway, HTLV-I infection, and inflammatory bowel disease were the top 5 enriched KEGG categories (**Fig. 3B**).

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Table 1. Antibody array results for significantly changed protein in TNCB 8 weeks treated group based on fold-change rank

Rank (up-regulated)	Antibody name	Fold-change	Gene symbol	Swiss-Prot entry
1	IL-9	4.977	IL9	P15247
2	Dtk	4.792	TYRO3	P55144
3	FGFR3	4.016	FGFR3	Q61851
4	IL-1 Ra	3.934	IL1R1	P13504
5	IL-12p70	3.841	IL12A/B	P43432, P43431
6	GFR alpha-4/GDNF R alpha-4	3.547	GFRA4	Q9JJT2
7	Endostatin	3.498	COL18A1	P39061
8	IL-27	3.421	IL27	Q8K3I6
9	IL-22	3.314	IL22	Q9JJY9
10	Glut2	3.270	SLC2A2	P14246
11	Common gamma chain/IL-2 R gamma	3.226	IL2RG	P34902
12	IL-1 R4/ST2	3.221	IL1RL1	P14719
13	ICAM-2/CD102	3.098	ICAM2	P35330
14	CRP	3.011	CRP	P14847
15	bFGF	3.011	FGF2	P15655
16	Soggy-1	2.978	DKKL1	Q9QZL9
17	CTACK	2.952	CCL27	Q9Z1X0
18	CXCR6	2.896	CXCR6	Q9EQ16
19	IL-11	2.865	IL11	P47873
20	Frizzled-6	2.865	FZD6	Q61089
21	TCA-3	2.854	CCL1	P10146
22	VE-cadherin	2.823	CDH5	P55284
23	IL-16	2.804	IL16	O54824
24	ICK	2.677	ICK	Q9JKV2
25	Lymphotoxin beta R/TNFRSF3	2.665	LTBR	P50284
26	IFN-beta	2.645	IFNB1	P01575
27	CCL28	2.643	CCL28	Q9JIL2
28	ICAM-1	2.603	ICAM1	P13597
29	TGF-beta RII	2.594	TGFBR2	Q62312
30	CCL1/I-309/TCA-3	2.521	CCL1	P10146
31	IL-23 R	2.519	IL23R	Q8K4B4
32	IL-17 R	2.508	IL17RA	Q60943
33	IL-28/IFN-lambda	2.496	IL28B	Q8CGK6
34	Prolactin	2.459	PRL	P06879
35	LIX	2.456	CXCL5	P50228
36	Follistatin-like 1	2.407	FSTL1	Q62356
37	VEGF-B	2.379	VEGFB	P49766
38	Decorin	2.376	DCN	P28654
39	CXCL14/BRAK	2.334	CXCL14	Q9WUQ5
40	AgRP	2.306	AGRP	P56473
41	IL-15 R alpha	2.284	IL15RA	Q60819
42	Eotaxin-2	2.251	CCL24	Q9JKC0
43	Pentraxin3/TSG-14	2.250	PTX3	P48759
44	IGFBP-3	2.221	IGFBP3	P47878
45	WISP-1/CCN4	2.179	WISP1	O54775
46	SPARC	2.149	SPARC	P07214
47	GDF-8	2.130	MSTN	O08689
48	HVEM/TNFRSF14	2.069	TNFRSF14	NP_849262
49	EG-VEGF/PK1	2.068	PROK1	NP_001037847
50	TCCR/WSX-1	2.060	IL27RA	O70394
51	TLR2	2.050	TLR2	Q9QUN7
52	TWEAK R/TNFRSF12	2.030	TNFRSF12A	Q9CR75
53	PF-4	2.003	PF4	Q9Z126

TNCB, trinitrochlorobenzene; IL, interleukin.

In the GO_BP results for the HDM-treated group, 30 components among the list of proteins that were regulated by HDM were significantly enriched. Among them, the top 10 enriched GO_BP terms were immune response, positive regulation of tyrosine phosphorylation of

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Table 2. Antibody array results for significantly changed protein in HDM treated group based on fold-change rank

Rank	Antibody name	Fold-change	Gene symbol	Swiss-Prot entry
Up-regulated				
1	IL-12p70	3.112	IL12A/B	P43432, P43431
2	MIP-1gamma	2.322	CCL9	P51670
3	NOV/CCN3	2.302	NOV	Q64299
4	Thymus chemokine-1	2.210	PPBP	Q9EQI5
5	RAGE	2.027	RAGE	Q9WVS4
Down-regulated				
1	IL-9	0.169	IL9	P15247
2	Dtk	0.219	TYRO3	P55144
3	FGF R3	0.347	FGFR3	Q61851

HDM, house dust mite; IL, interleukin.

STAT4 protein, positive regulation of mononuclear cell proliferation, positive regulation of NK T cell activation, positive regulation of natural killer cell-mediated cytotoxicity directed against tumor cell target, response to UV-B, positive regulation of lymphocyte proliferation, positive regulation of the smooth muscle cell apoptotic process, positive regulation of natural killer cell activation, and negative regulation of interleukin (IL)-17 production (**Fig. 3C**). Among the KEGG results, there were 15 pathways that were significantly enriched in the HDM-treated group. Among them, cytokine-cytokine receptor interaction, Jak-STAT signaling pathway, African trypanosomiasis, allograft rejection, and legionellosis were the top 5 enriched KEGG categories (**Fig. 3C**).

Comparison of skin lesion scores between groups

Clinical symptoms including erythema/hemorrhage, scarring/dryness, edema, and excoriation/erosion were most serious in the TNCB 2 weeks-treated group (**Fig. 4A**). The scoring of skin lesions among the groups showed significant differences ($p = 0.0007$). The skin lesion scoring results in the TNCB 2 weeks-treated group ($p = 0.0097$), TNCB 8 weeks-treated group ($p = 0.0097$) and the HDM-treated group ($p = 0.0097$) were significantly different from that of the control group. Moreover, the skin lesion scores in the TNCB 8 weeks-treated group ($p = 0.0112$) and the HDM-treated group ($p = 0.0112$) were significantly different from that in the TNCB 2 weeks-treated group. However, a comparison of the skin lesion scores between the TNCB 8 weeks-treated group and the HDM-treated group failed to detect a significant difference ($p = 0.136$) (**Fig. 4B**).

Comparison of scratching frequencies between groups

The scratching frequencies of the groups were significantly different ($p = 0.0007$). The scratching frequencies in the TNCB 2 weeks-treated group ($p = 0.0097$), TNCB 8 weeks-treated group ($p = 0.0097$) and HDM-treated group ($p = 0.0097$) were significantly different from that of the control group. Moreover, the scratching frequencies in the TNCB 8 weeks-treated group ($p = 0.0119$) and the HDM-treated group ($p = 0.0119$) were significantly different from that of TNCB 2 weeks-treated group. In addition, a comparison of the scratching frequencies of the TNCB 8 weeks-treated group HDM-treated groups showed the presence of a significant difference ($p = 0.0112$) (**Fig. 4C**).

Comparison of histological results between groups

The H&E staining of control group tissue revealed normal structures within the epidermis, dermis, subcutaneous layer, and muscle layer. Compared to the control group tissue, epidermal and dermal hyperplasia, excessive keratinization, and infiltration of lymphocytes were exhibited in the TNCB 2 weeks-treated group. In the TNCB 8 weeks-treated and HDM-treated groups,

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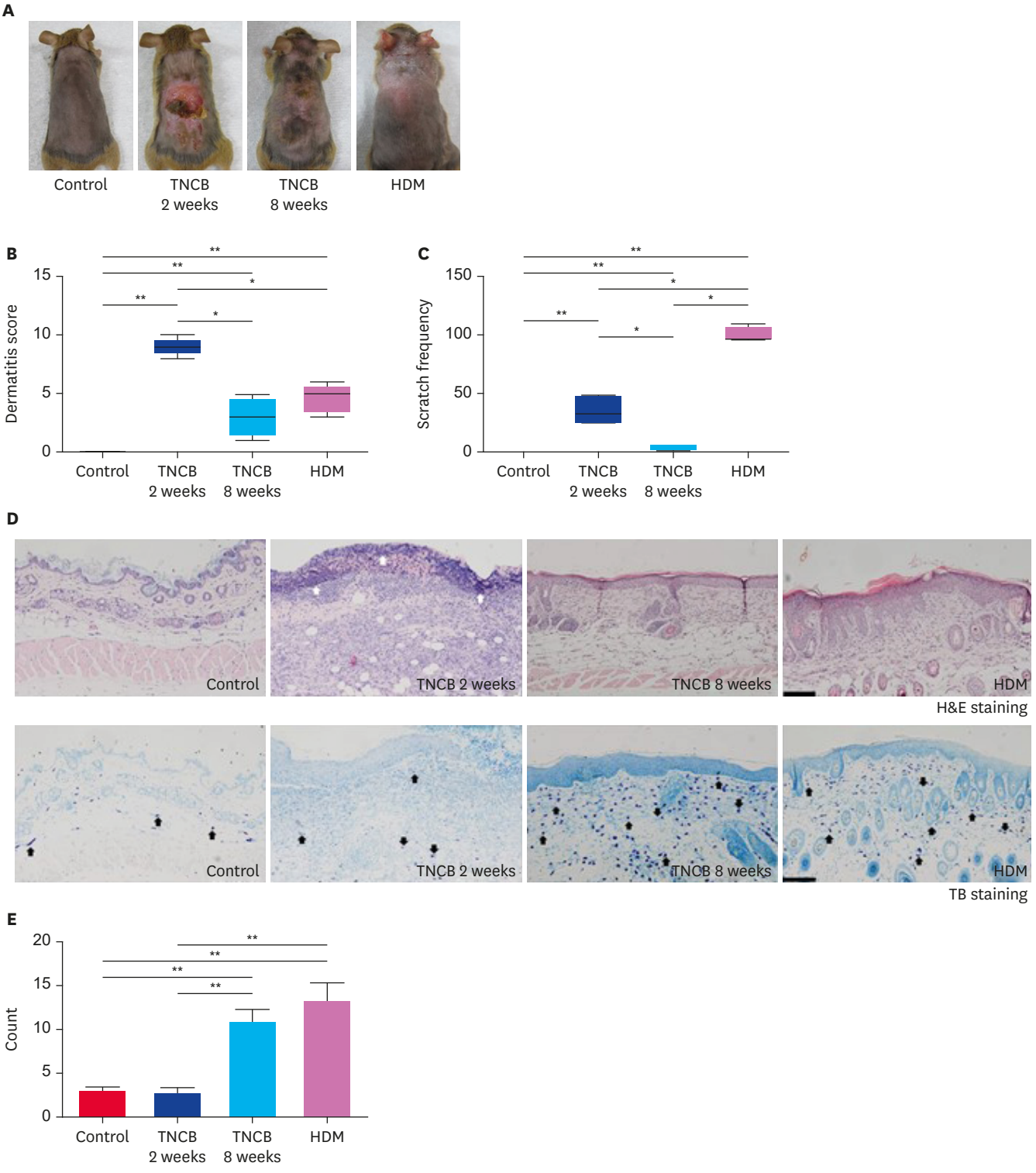

Fig. 4. (A) Representative clinical features in control, TNCB 2 weeks-treated, TNCB 8 weeks-treated and HDM-treated groups. (B) Dermatitis scores of the groups were significantly different except the scores of the TNCB 8 weeks-treated and HDM-treated groups were similar. (C) Scratching frequencies of the groups were significantly different. (D) Inflammatory cells (white arrow) were excessively exhibited in the TNCB 2 weeks-treated group. Mast cells (black arrow) in the dermis were excessively exhibited in the TNCB 8 weeks-treated group. Scale bar indicated 250 μm. (E) The mast cell density was expressed as the number of cells per 250 μm^2 for each section. The significantly different was indicated between groups of the mast cells density.
TNCB, trinitrochlorobenzene; HDM, house dust mite.
$^*p < 0.05$; $^{**}p < 0.01$.

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there was skin damage from the epithelium to the dermis, but the infiltration of lymphocytes was less than that exhibited by the TNCB 2 weeks-treated group tissues (**Fig. 4D**).

The TB staining results showed that the number of mast cells in the dermis was higher in the TCNB 8 weeks-treated (p = 0.0005, 0.0006) and HDM-treated groups (p = 0.0004, 0.0004) than that in the dermis of the control group and TNCB 2 weeks-treated group, each (**Fig. 4D and E**).

DISCUSSION

There have been many studies into establishing AD models [8-10] useful in elucidating the pathologies and development of AD. However, there are few reports comparing AD models. In this study, we established Nc/Nga mouse AD models that were induced with either TNCB for short (2 weeks) and long (8 weeks) periods or with HDM and compared the effects in each of the groups.

Recently, the possibility that repeated application of haptens, such as TNCB or oxazolone, over an extended period can cause skin inflammation to shift from a typical Th1-dominated delayed-type hypersensitivity response to a chronic Th2-dominated inflammatory response has been suggested [10,19]. In this study, we observed notably different results between the short and long periods of TNCB treatment. Even though the serum IgE concentration, which was produced by B cells, was not significantly different between TNCB treatment periods, cytokine antibody array analysis, dermatitis score, and scratching frequency were significantly different between the short and long TNCB treatment periods. Moreover, antibody array analysis, which analyzed 308 cytokines, showed there were no cytokines exhibiting fold changes greater than 2 (compared to the control group) in the TNCB 2 weeks-treated group. However, in the comparison of the TNCB 8 weeks-treated group and the control group, there were 53 significantly up-regulated proteins following the long period (8 weeks) of TNCB treatment.

Among the significantly up-regulated proteins, the expression of IL-9 was the highest observed in the TNCB 8 weeks-treated group. IL-9 is a pleiotropic cytokine (cell signaling molecule) produced by various cells, including mast, NK T, Th2, Th9, Th17, and regulatory T cells. In combination with the IL-9 receptor, it exerts a variety of biological functions through the STAT pathway [20]. There are diverse opinions about the effect of IL-9 expression in AD. Previous studies have described the proliferation of Th9 cells and the expression of IL-9 in allergic respiratory diseases, asthma, and rhinitis [21]. In one study, observation of increasing expression of IL-9 and proliferation of Th9 cells in human AD suggested that they can perform the potential roles of Th2 cells in AD [22]. Similarly, another study showed that the serum IL-9 level in patients with AD was higher than that in normal human children [23]. On the other hand, there was a study that showed a decrease in the IL-9-enhanced Th1 response in AD [21]. The results of cytokine array analysis in the present study support the hypothesis that TNCB treatment over an extended period can cause skin inflammation to shift from a typical Th1-dominated delayed-type hypersensitivity response to a chronic Th2-dominated inflammatory response [10,19].

The studies related HDM induced AD in Nc/Nga mice could not solve the pathogenesis clearly. They confirmed that application of a HDM extract to the skin of Nc/Nga mice can

increase the clinical, histological symptoms and serum IgE concentration [15,24,25]. One of the study showed that the expressions of Th1/Th2/Th17 related cytokines were increased in HDM induced AD in Nc/Nga mice [15]. In this study, we confirmed similar results with the previous studies. Interestingly, even though the patterns of serum IgE concentrations, skin lesion scores, and histological results in the TNCB 8 weeks-treated group were similar to the patterns in the HDM-treated group, the expression of IL-9, based on cytokine array analysis, was lowest in HDM-treated group. We assumed that the AD patterns induced by repeated long-period TNCB treatment would be similar with the AD patterns induced by HDM treatment based on the serum IgE concentration, scoring of skin lesions, and histological analysis results, but are unable to explain the inflammatory response to the HDM treatment because IL-9 was the lowest in the HDM-treated group.

The scratch scoring results showed that scratch frequency was the highest in the HDM treatment group, although the serum IgE concentration was the highest in the TNCB 2 weeks-treated group. Based on these results, we assume that HDM induced scratching behavior via IgE-independent mechanisms. Yamada et al. [15] also observed that HDM-induced scratching behavior was not related to IgE concentration.

Based on this study, we confirm that the immunological patterns of each AD-induced animal model group were different; even treatment duration could produce a different immune response in an AD-induced animal model.

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