Lab. Anim. Res. 2010: 26(1), 95-102

Laboratory Animal Research

http://submission.kalas.or.kr

A Novel Model for Human Atopic Dermatitis: Application of Repeated DNCB Patch in BALB/c Mice, in Comparison with NC/Nga Mice

Kyoung-Sun Lee, Eui-Suk Jeong, Seung-Ho Heo, Jin-Hee Seo, Dong-Gu Jeong and Yang-Kyu Choi*

Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University, Seoul, Korea

The various murine models have contributed to the study of human atopic dermatitis (AD). However limitations of the models involve low reproducibility and long time to develop AD. In an attempt to overcome these limitations and establish an atopic dermatitis murine model, we repeated the application of 2, 4-dinitrochlorobenzene (DNCB) patch in NC/Nga and BALB/c mice, which has advantages in reproduction and cost. For the sensitization, a 1 cm^2 gauze-attached patch, where 1% or 0.2% DNCB was periodically attached on the back of NC/Nga and BALB/c mice. To estimate how homologous our model was with human atopic dermatitis, clinical, histological and immunological alterations were evaluated. Both strains showed severe atopic dermatitis, increase in subiliac lymph node weight, mast cells, epidermal hyperplasia and serum IgE levels. Though both exhibited a high IL-4/IFN-γ and IL-4/TNF-β ratio in the expression of mRNA, the shifting of DNCB-treated BALB/c mice was increased to more than double that of NC/Nga mice. These results suggest that our DNCB patched model using BALB/c mice were more suitable than NC/Nga mice in demonstrating the immune response. We anticipate that our novel model may be successfully used for pathogenesis of atopic dermatitis and assessment of therapeutic approaches.

Key words: Atopic dermatitis, BALB/c, 2,4-dinitrochlorobenzene (DNCB), NC/Nga

(Received 2 March 2010; Revised version received 15 March 2010; Accepted 17 March 2010)

Atopic dermatitis among common skin diseases is a chronic and recurrent inflammatory skin disorder caused by genetic, environmental, allergens as well as microbial factors (Sugiura *et al.*, 2003; Novak and Bieber, 2005). 2. Atopic dermatitis patients clinically present skin erythematous plaques, eruption, elevated serum IgE and Th2 cytokine levels, such as IL-4 and IL-13. Microscopically, atopic dermatitis patients also show epidermal hyperplasia and accumulation of mast cells and T helper cell type 2 (Th2) (Akdis *et al.*, 2006; Novak, 2009). Though many possible mechanisms of atopic dermatitis have been researched and suggested, its etiology remains unclear.

Recently, many investigators have used various murine models to conduct an *in vivo* study on atopic dermatitis. These models were classified into two groups (Marsella and Olivry, 2003; Jin *et al.*, 2009); (a), spontaneous mutants and genetically engineered mutants, such as NC/Nga mice and IL-4/18-overexpressing mice, (b), sensitizer-induced models, using ovalbumin, microbial antigen (mite or *staphylococcus aureus*) and chemical reagents (haptens; picryl chloride, trinitrochlorobenzene, 2,4-dinitrochlorobenzene or oxazolone). Among these, the murine model of repeated hapten applications possesses benefits of reproducibility and economic viability (Man *et al.*, 2008). Painted repeatedly on the skin of mice, the hapten allowed the conversion of the immune response from a Th1 to Th2-dominated response and also induced high serum IgE levels, epidermal hyperplasia and infiltration of mast cell in dermis, all of which are regarded as particular features of human atopic dermatitis (Kitagaki *et al.*, 1995; Kitagaki *et al.*, 1997).

Immune responses vary based on the different strains of mice studied. For instance, the BALB/c strain is biased toward the Th2 response, while the C57BL/6 strain displays a Th1 dominant response. Based on such immunological differences, some reports developed murine models of atopic dermatitis in BALB/c mice, sensitized repeatedly by albumin and picryl chloride (Inoue *et al.*, 2002; Yatsuzuka *et al.*, 2007). However,

*Corresponding author: Yang-Kyu Choi, Department of Laboratory Animal Medicine, College of Veterinary Medicine, Konkuk University, #1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, South Korea
Tel: +82-2-2049-6113
Fax: +82-2-453-6113
E-mail: yangkyuc@konkuk.ac.kr

these models required a relatively longer time for atopic dermatitis induction. Another atopic dermatitis models using various haptens are difficult to paint the haptens on same size in each mouse (Taniguchi *et al.*, 2003; Lee *et al.*, 2006; Sunada *et al.*, 2008). Different sizes cause the different concentration of sensitizer per area and induce the different levels of sensitization of the skin (Friedmann, 2006). Furthermore, intake of the haptens by the mice is not easy to prevent. Those limitations weaken the experimental accuracy and data reproducibility. To supplement such weaknesses and develop a more convenient and reproducible model, we used a 2,4-dinitrochlorobenzene (DNCB) and patch in BALB/c and NC/Nga mice. Then, we estimated how homologous our models was with human atopic dermatitis via clinical, histological and immunological alterations.

Materials and Methods

Animals and reagents

Male BALB/c and Nc/Nga mice were purchased from Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea) and SLC (Shizuoka, Japan) and were 7 weeks olds at the initiation of the study. All mice were maintained at the barrier facility of the College of Veterinary Medicine at Konkuk University (Seoul, Korea) and were housed on woodchip bedding (Sani-chip®; Harlan TEKLAD, Madison, WI, USA) with a light-dark cycle of 12:12 h (08:00 to 20:00 h). The room temperature was maintained at 22±2°C with a relative humidity of 50±10%. The animals were fed a sterilized pelleted diet (2918C®; Harlan TEKLAD, Madison, WI, USA) and had access to autoclaved water through drinking bottles *ad libitum*. The study was conducted using animal protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University. 2,4-dinitrochlorobenzene used as a sensitizer was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in acetone-olive oil (BioBasic Inc., Toronto, Ontario, Canada) in a ratio of 4:1. The acetone-olive oil (AOO) was used as vehicle.

Sensitization and challenge

The backs of mice were shaved with an electric clipper and depilatory cream, and washed with sterilized PBS-gauzed a day before sensitization. For the sensitization process (Figure 1), a-cm^2 gauze-attached patch (Tegaderm®; 3M Health Care, MN, USA), applied with 0.1 mL of 1% DNCB in vehicle or vehicle alone was attached to the shaved backs of the animals for 2 day on day 0 and 3. On day 7 and 10, 0.1 mL of 0.2% DNCB in vehicle or vehicle were used to challenge for a day as a previous sensitization.

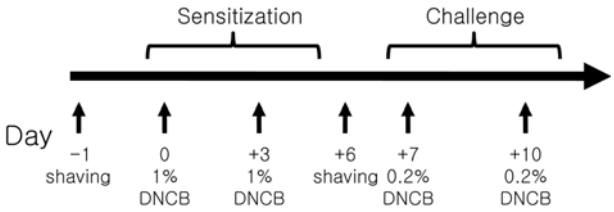

Figure 1. Schematic diagram of the study protocol. The back of NC/Nga mice and BALB/c were shaved with electric clipper and depilatory cream. 1 cm^2-gauze treated 0.1 mL of 2% DNCB and vehicle was placed on a patch, which was attached to the mice twice a week for sensitization. After sensitization, 0.1 mL of 0.2% DNCB and vehicle were used to challenge the animals as the previous sensitization.

Evaluation of skin lesion

The severity of dermatitis was clinically assessed using previously established methods (Matsuda *et al.*, 1997; Kim *et al.*, 2008). The total scores of skin severity were defined as a sum of the individual score (0, no symptoms; 1, mild; 2, moderate; 3, severe) for each of the following four signs and symptoms: erythema/hemorrhage; edema; excoriation/erosion; dryness. The total dermatitis score was defined as the individual scores indicated above (maximum score: 12).

Histopathological analysis

For histopathological examinations, skin samples from the back of each mouse were prepared in 10% neutral buffered formalin, embedded in paraffin and cut into four-micrometer-thick sections. The sections were stained with hematoxylin-eosin (HE) to evaluate epidermal hyperplasia and infiltration of immune cells in the dermis, as well as toluidine blue to count the number of mast cells in five random fields (×400) using a computerized image analyzer (MetaMorph 7.5; Molecular Devices, Downingtown, PA, USA).

Measurement of serum IgE levels

Mice were anesthetized by intra-peritoneal injection of tribromoethanol (125-250 mg/kg, Sigma-Aldrich). Blood samples were obtained from the retro-obital venous plexus of mice and serum was separated via centrifugation and stored at −80°C until tested. The assessments of IgE were performed using enzyme-linked immunosorbent assay kits (BD OptEIA®; BD Pharmingen, San Diego, CA, USA) according to the manufacturer's protocol. Briefly, the capture antibodies were added to each well in a 96-well ELISA plate (Costar®; Corning Inc., Corning, NY, USA) and incubated to adhere overnight at 4°C. The plates were washed and then blocked with 10% fetal bovine serum in phosphate-buffered saline for 1 h at room temperature (RT). After washing, the diluted standard and samples were added in the plates and incubated for

Table 1. cytokine-specific primer pair sequences used in RT-PCR

Cytokine	Oligonucleotide sequences (5'-3')	Size(bp)	Tm (°C)	Cycle
β-actin	F: CCC TGA AGT ACC CCA TTG AA R: GGG GTG TTG AAG GTC TCA AA	193	55	20 & 23
IL-4	F: CGG ATG CGA CAA AAA TCA R: CTT ATC GAT GAA TCC AGG CA	270	60	30 & 35
TNF-α	F: AAC TAG TGG TGC CAG CCG AT R: CTT CAC AGA GCA ATG ACT CC	333	60	25 & 30
IFN-γ	F: AAC GCT ACA CACTGC ATC TT R: GCT GGA CCT GTG GGT TGT	379	60	32 & 35

2 h at RT. After washing and adding 100 μL of working detector (including detector antibody and avidin-HRP reagent) to each well, the plates were incubated for 1 h at RT and then washed. The substrate solution (0.1 mL) was transferred into each well, incubated in the dark for 30 mins at RT and absorbance was read at 450 and 570 nm. IgE levels in serum were quantified by comparison to the standard.

Semi-quantitative RT-PCR for cytokine-specific mRNA

For detection of cytokine-specific mRNA (IFN-γ, TNF-α and IL-4), total RNA samples were prepared from the skin of DNCB or vehicle-treated mice using the One Step-RNA® reagent (BioBasic Inc., Toronto, Canada) by following the manufacturer's directions. Aliquots of total RNA (2 mg) were reverse transcribed. A microliter volume of cDNA prepared as described above was used for PCR amplification with PCR premix (Hotstart®; Bioneer Inc., Daejeon, Korea) at two different cycles, unsaturated. Each primer pair sequence and condition was designed on the basis of published gene sequences as indicated in Table 1. The PCR products were electrophoresed on a 1.5 % agarose gel and photographed under UV transillumination. For the quantitative analysis, the mean band intensities in two cycles of each cytokine were normalized to that corresponding β-actin using a densitometer (Multi Gauge v3.0 software; Fujifilm Life Science, Tokyo, Japan).

Statistical analysis

All values are presented as means±SD. $P<0.05$ was considered to be significant in all statistical tests. Data were compared between groups. One-way ANOVA (SPSS 12.0K for Windows, SPSS Institute Inc., USA) was used to compare all pairs of means at the point of same time.

Results

Induction of atopic dermatitis by repeated-application of DNCB

To determine whether atopic dermatitis-like skin lesions were developed by repeated application of DNCB, clinical and histopathological analysis was performed on the back of mice. As shown in Figure 2, the dermatitic lesions of DNCB-treated groups increased significantly, while vehicle-treated groups demonstrated no change. We next performed histopathological analysis using hematoxylin-eosin and toluidine blue stains (Figure 3). The skins of DNCB-treated

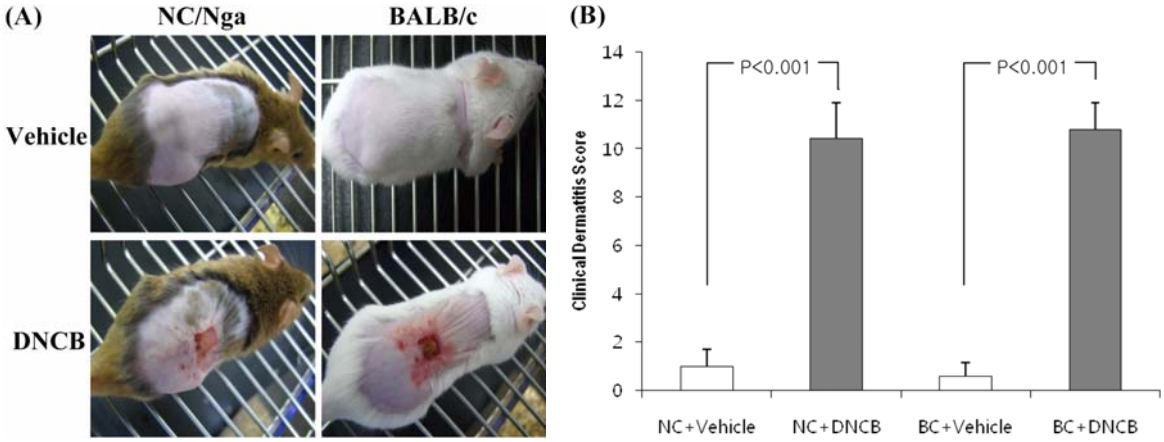

Figure 2. (A) Pictures of the clinical observations on the back of mice. (B) Evaluation of clinical dermatitis induced by DNCB and vehicle. The evaluation of clinical dermatitis was based on the areas of gauze which delivered the DNCB and vehicle. Each column shows the mean±SD of 5 mice.

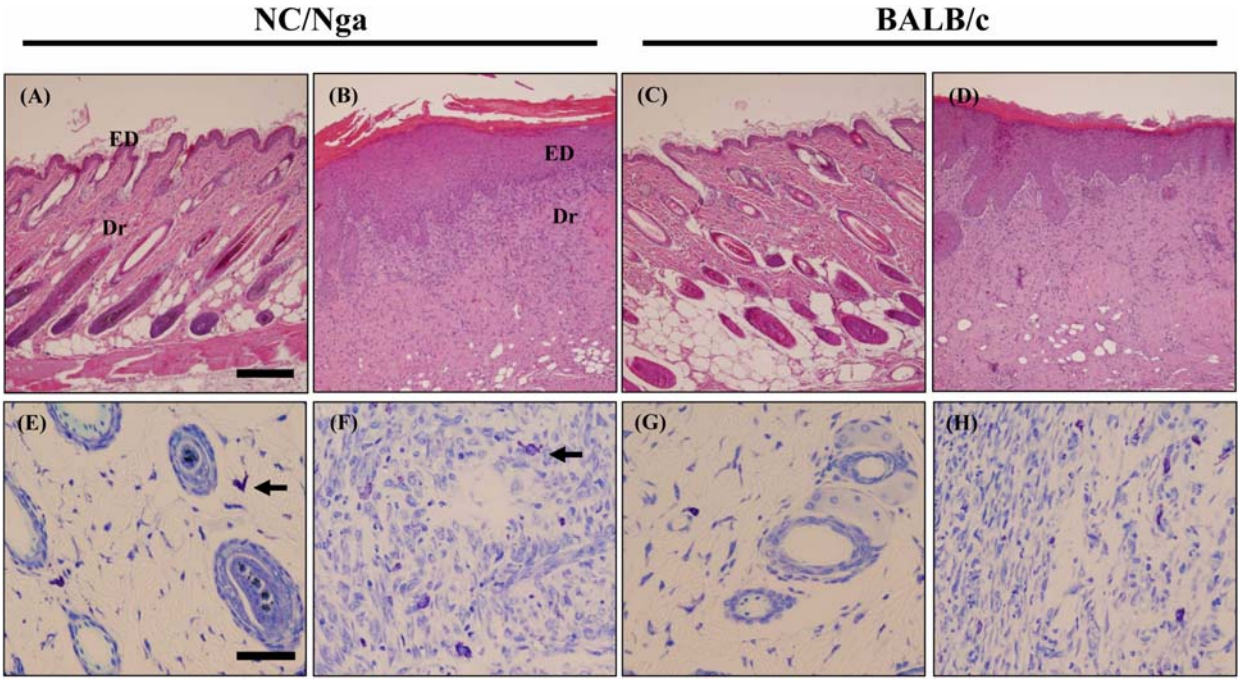

Figure 3. Histopathological alterations by DNCB and vehicle. Skins of mice were stained with hematoxylin & eosin (×100, A-D) and toluindine blue (×400, E-H). (A, C, E, G) are vehicle-treated groups and (B, D, F, H) are DNCB-treated groups. ED, epidermis; Dr, dermis; arrow, mast cells. Bar in ×100=200 μm, Bar in ×400=50 μm.

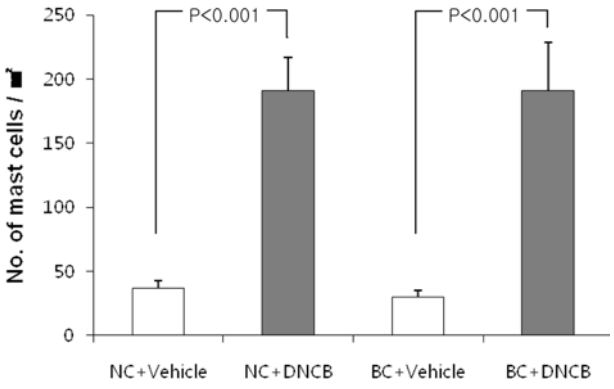

Figure 4. Quantification of mast cells in dorsal skin of mice treated with DNCB and vehicle. Skin of mice were stained with toluidine blue and the numbers of mast cell in the dermis of the skin were quantitatively analyzed in 5 high power fields (×400) by a computerized image analyzer. Each column shows the mean±SD of 5 mice.

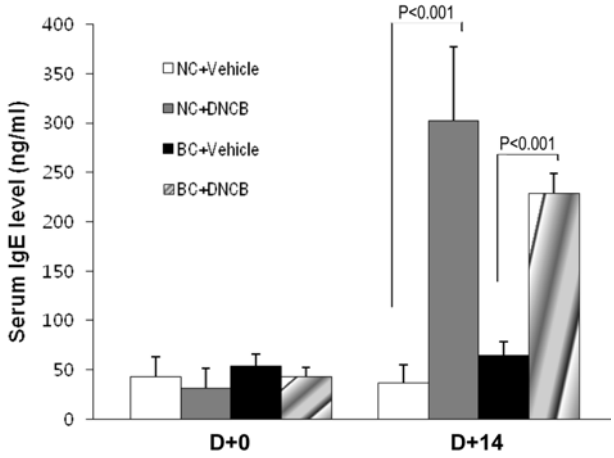

Figure 5. Changes in serum total IgE levels. Blood samples were collected from retro-obital venous plexus before treatment with DNCB and at 4 days after the last treatment. Serum IgE was quantified using ELISA. Each column shows the mean± SD of 4 mice.

groups distinctly showed epidermal hyperplasia and infiltration of inflammatory cells, including lymphocytes and eosinophils, in the dermis, unlike the vehicle-treated groups. Also, the number of mast cells in DNCB-treated groups was significantly higher than that in vehicle-treated groups (Figure 4). These results indicate that repeated application of DNCB induced epidermal hyperplasia and recruited inflammatory cells to the dermis of the applied site without strain differences between NC/Nga and BALB/c mice.

Increase in serum IgE by DNCB treatment

We investigated the changes of serum IgE in our models (Figure 5). Apparent elevation of serum IgE is known as the one of the most characteristic features of atopic dermatitis patients. Compared with the vehicle groups, the serum IgE levels of DNCB-treated groups were increased markedly. Though NC/Nga mice showed the higher mean of serum IgE than BALB/c mice, the difference were no statistical significance.

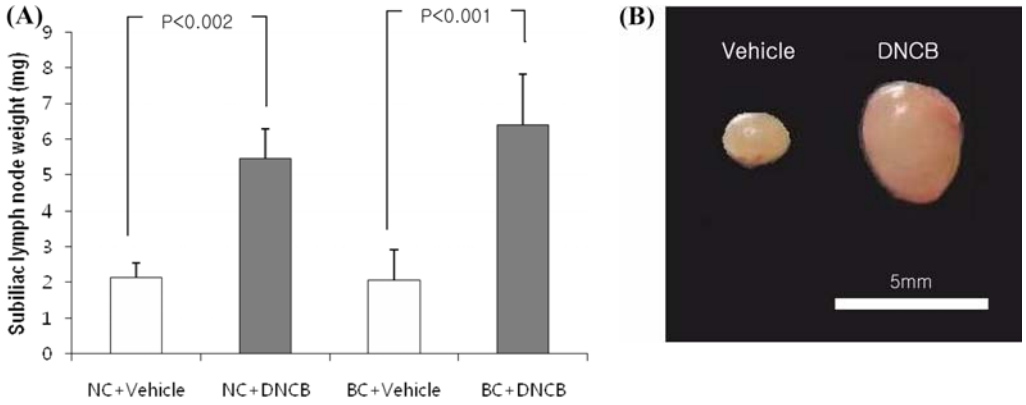

Figure 6. Changes in subiliac lymph node weight induced by DNCB and vehicle. The subiliac lymph node approximates to the site, treated by DNCB and vehicle. The weights of subiliac lymph node were measured 8 days after the last treatment. Each column shows the mean±SD of 5 mice.

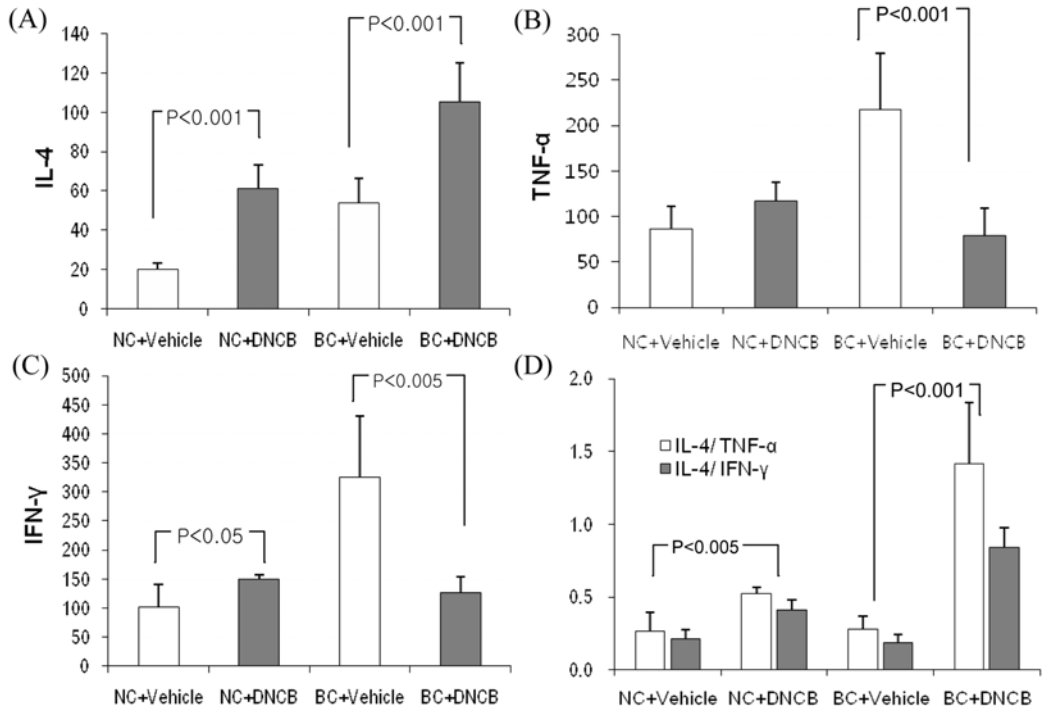

Figure 7. Expression of IL-4, IFN-γ and TNF-α mRNA levels in skin painted repeatedly with DNCB and vehicle. Total mRNA was extracted from skin and reverse-transcribed as described in materials and methods. IL-4 (A), TNF-α (B) and IFN-γ (C) mRNA expression were normalized with β-actin. (D) IL-4/TNF-α (white bars) and IL-4/IFN-γ mRNA (black bars). Each column shows the mean±SD of 5 mice.

Increase in local lymph node weight by DNCB treatment

A common method for the identification of skin-sensitizing materials is the murine local lymph node assay, which evaluates the proliferative responses of local lymph node by locally applied materials (Kimber *et al.*, 2001; Kang *et al.*, 2006). Thus, the weight of the local lymph node informs us about a certain magnitude of sensitization by DNCB. Figure 6 shows that the weight of the subiliac lymph node was higher in DNCB-treated NC/Nga and BALB/c mice. This result means DNCB caused proliferation of the lymphocyte in the lymph node.

Changes of cytokine mRNA expression following skin exposure to repeated application of DNCB and vehicle

The Th2-dominant immune response plays a critical role in developing atopic dermatitis. To evaluate the immune response in the skin of our animal models, we studied the expression of IL-4, IFN-γ and TNF-α mRNA (Figure 7). The

skins of DNCB-treated groups induced higher IL-4 mRNA expression than that of vehicle-treated groups (Figure 7A). The levels of both Th1 cytokines, IFN-γ and TNF-α, in only DNCB-treated BALB/c mice were decreased apparently, while DNCB-treated NC/Nga mice increased (Figure 7B & 7C).

There was an increase in the IL-4/IFN-γ and IL-4/TNF-α mRNA ratio (Figure 7D) in both DNCB-treated strains. The increase caused by DNCB treatment was more distinct in BALB/c mice than in NC/Nga mice. These results indicated a more severe shift toward Th2- dominant response after repeated application of DNCB in BALB/c mice than NC/Nga mice.

Discussion

Recently, the prevalence of atopic dermatitis has increased two- and threefold around the world, especially in industrialized countries. The onset of atopic dermatitis usually occurs during early infancy and childhood, but it can persist into or start in adulthood (Leung *et al.*, 2004). Also, patients with atopic dermatitis are at a higher risk of developing asthma, food allergy and allergic rhinitis (Spergel *et al.*, 1998; Gustafsson *et al.*, 2000).

NC/Nga mice have been well-established as a spontaneous animal model for human atopic dermatitis, due to the impaired chromosome 9. But, various limitations, such as low incidence (<50%) and late onset of atopic dermatitis-like lesions, were discovered during atopic dermatitis studies using NC/Nga mice (Shiohara *et al.*, 2004). Though the additional applications of haptens or mite antigen (Kang *et al.*, 2006) on NC/Nga mice is possible, these mice have great disadvantages as an atopic dermatitis model due to long induction time and difficult sensitization results even when using the same concentration of allergens. Another well-known model for atopic dermatitis is the use of transgenic mice overexpressing IL-4, IL-18, IL-31 and Caspase-1, which is useful for pathogenic studies but not for therapeutic studies (Yamanaka *et al.*, 2000; Chan *et al.*, 2001; Tanaka *et al.*, 2001; Dillon *et al.*, 2004). In addition, these mice require a long induction time and commercial exploitation is difficult. Therefore, an inexpensive, reproducible and shortly-inducible model was needed.

In the present study, we established an atopic dermatitis model by the repeated application of a DNCB-patch in BALB/c mice, and compared this model to the NC/Nga mice. The patch is a useful method of applying DNCB to the same location at the same size. Our model was shown to be more cost-effective and more reproducible than previous models because an equivalent concentration of sensitizer per area was successfully applied without any loss, intake and leakage.

Clinical and histopathological lesions in human atopic dermatitis show erythema, dryness, epidermal hyperplasia and the pronounced infiltration of lymphocyte and mast cells (Navi *et al.*, 2007; Wollenberg and Klein, 2007). Herein, clinical lesions in both DNCB-treated groups were similar to human atopic dermatitis (Figure 2); histopathological analysis showed the presence of hyperkeratosis, a thick epidermis, and the infiltration of lymphocyte and mast cells dermis (Figures 3, 4). Mast cells can be sensitized by the high affinity IgE receptors (FcεRI) on the cell surface and have also been involved in the key effector cells. Mast cells are related to the Th2 response of atopic dermatitis skin. Some reports demonstrated that 66% of mast cells express IL-4 in the lesional skin of atopic dermatitis patients and induce Th2 polarization of skin (Horsmanheimo *et al.*, 1994; Theiner *et al.*, 2006). In the DNCB-treated groups, the increased infiltration of mast cells was similar to that of human atopic dermatitis. The high level of serum IgE helps mast cells sensitize and, in turn, changes into Th2-skewed immune responses. In present study the elevated level of serum IgE in both DNCB-treated strains was lower than that of other hapten-induced models (Guo *et al.*, 2002; Kim *et al.*, 2008; Sunada *et al.*, 2008). This may be a result of differently applied areas, consumption of the hapten by the animal, exposal period or chemical difference.

In present study, both DNCB-treated strains presented higher IL-4/IFN-γ and IL-4/TNF-α m RNA ratios than the vehicle-treated groups (Figure 7). Many investigators agree that the imbalance of Th1 and Th2 cytokines play an important role in the immunological pathway of atopic dermatitis. T cells, antIgEn-presenting cells and keratinocytes are regarded as key effector cells in atopic dermatitis (Leung *et al.*, 2004; Akdis *et al.*, 2006). Compared to the normal skin of atopic dermatitis patients, lesional skin exhibits a greater number of Th2 cytokines, IL-4 and IL-5. The former induces the switching signals on B-cell for IgE synthesis and initiates acute skin inflammation in atopic dermatitis, while the latter engages in eosinophil development and survival and maintains chronic inflammation (Hamid *et al.*, 1994; Akdis *et al.*, 2006). In addition, they inhibit the differentiation of naïve T-cells toward Th1 and the production of human beta defensin (HBD)-3, an antimicrobial peptide induced by TNF-α and IFN-γ (Opal and DePalo, 2000; Nomura *et al.*, 2003). Therefore, the increased IL-4/IFN-γ and IL-4/TNF-α mRNA ratio by DNCB treatment demonstrated the induction of Th2-dominant response and was associated with the high level of serum IgE (Figure 5).

In the mRNA expression of vehicle-treated groups, an interesting finding was the apparent increase in IL-4 mRNA

levels of BALB/c mice in comparison to that of NC/Nga mice (Figure 7A). However, the IL-4/IFN-γ and IL-4/TNF-α mRNA ratios showed no difference between NC/Nga and BALB/c mice (Figure 7D), because the Th1 cytokines were increased relatively (Figures 7B & 7C). This indicated that the immune response of BALB/c mice was biased toward Th2, which was compensated for by the relative increase of Th1 cytokine. Consequently, this homeostasis keeps the balance of the immune response in vehicle-treated groups. However, the repeated applications of DNCB broke the balance of Th1 and Th2, and prompted a shift toward the Th2- dominant response. Comparing with the shifting of DNCB-treated NC/Nga mice, BALB/c mice were increased by more than double. These results indicated the Th2-skewed immune responses by DNCB were clearly different depending on strains.

In conclusion, using DNCB-patches in BALB/c and NC/Nga mice, we applied same concentration of DNCB on same area. Though both DNCB-treated strains showed typical atopic dermatitis lesion, BALB/c mice was more suitable than NC/Nga mice in demonstrating the immune response. This method in BALB/c mice appeared to be more convenient and reproducible than the previous models, and shortened the time taken to induce atopic dermatitis. Considering the cost and tendency toward TH2-skewed immune response, the BALB/c model is more advantageous than the NC/Nga model. Our model will be helpful for both atopic dermatitis pathogenesis and assessment of therapeutic approaches.

Acknowledgments

This work was supported by the Brain Korea 21 project funded by Korea Research Foundation.

References

Akdis, C.A., Akdis, M., Bieber, T., Bindslev-Jensen, C., Boguniewicz, M., Eigenmann, P., Hamid, Q., Kapp, A., Leung, D.Y., Lipozencic, J., Luger, T.A., Muraro, A., Novak, N., Platts-Mills, T.A., Rosenwasser, L., Scheynius, A., Simons, F.E., Spergel, J., Turjanmaa, K., Wahn, U., Weidinger, S., Werfel, T. and Zuberbier, T. (2006) Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. Allergy. 61, 969-987.

Chan, L.S., Robinson, N. and Xu, L. (2001) Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol. 117, 977-983.

Dillon, S.R., Sprecher, C., Hammond, A., Bilsborough, J., Rosenfeld-Franklin, M., Presnell, S.R., Haugen, H.S., Maurer, M., Harder, B., Johnston, J., Bort, S., Mudri, S., Kuijper, J.L., Bukowski, T., Shea, P., Dong, D.L., Dasovich, M., Grant, F.J., Lockwood, L., Levin, S.D., LeCiel, C., Waggie, K., Day, H., Topouzis, S., Kramer, J., Kuestner, R., Chen, Z., Foster, D., Parrish-Novak, J. and Gross, J.A. (2004) Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol. 5, 752-760.

Friedmann, P.S. (2006) Contact sensitisation and allergic contact dermatitis: immunobiological mechanisms. Toxicol Lett. 162, 49-54.

Guo, T.L., Zhang, X.L., Leffel, E.K., Peachee, V.L., Karrow, N.A., Germolec, D.R. and White, K.L., Jr. (2002) Differential stimulation of IgE production, STAT activation and cytokine and CD86 expression by 2,4-dinitrochlorobenzene and trimellitic anhydride. J Appl Toxicol. 22, 397-403.

Gustafsson, D., Sjoberg, O. and Foucard, T. (2000) Development of allergies and asthma in infants and young children with atopic dermatitis--a prospective follow-up to 7 years of age. Allergy. 55, 240-245.

Hamid, Q., Boguniewicz, M. and Leung, D.Y. (1994) Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. J Clin Invest. 94, 870-876.

Horsmanheimo, L., Harvima, I.T., Jarvikallio, A., Harvima, R.J., Naukkarinen, A. and Horsmanheimo, M. (1994) Mast cells are one major source of interleukin-4 in atopic dermatitis. Br J Dermatol. 131, 348-353.

Inoue, Y., Isobe, M., Shiohara, T., Goto, Y. and Hayashi, H. (2002) Protective and curative effects of topically applied CX-659S, a novel diaminouracil derivative, on chronic picryl chloride-induced contact hypersensitivity responses. Br J Dermatol. 147, 675-682.

Jin, H., He, R., Oyoshi, M. and Geha, R.S. (2009) Animal models of atopic dermatitis. J Invest Dermatol. 129, 31-40.

Kang, J.S., Lee, K., Han, S.B., Ahn, J.M., Lee, H., Han, M.H., Yoon, Y.D., Yoon, W.K., Park, S.K. and Kim, H.M. (2006) Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of Dermatophagoides pteronyssinus in NC/Nga mice. Int Immunopharmacol. 6, 1616-1622.

Kim, E.C., Lee, H.S., Kim, S.K., Choi, M.S., Lee, S., Han, J.B., An, H.J., Um, J.Y., Kim, H.M., Lee, N.Y., Bae, H. and Min, B.I. (2008) The bark of Betula platyphylla var. japonica inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. J Ethnopharmacol. 116, 270-278.

Kimber, I., Basketter, D.A., Berthold, K., Butler, M., Garrigue, J.L., Lea, L., Newsome, C., Roggeband, R., Steiling, W., Stropp, G., Waterman, S. and Wiemann, C. (2001) Skin sensitization testing in potency and risk assessment. Toxicol Sci. 59, 198-208.

Kitagaki, H., Fujisawa, S., Watanabe, K., Hayakawa, K. and Shiohara, T. (1995) Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol. 105, 749-755.

Kitagaki, H., Ono, N., Hayakawa, K., Kitazawa, T., Watanabe, K. and Shiohara, T. (1997) Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol. 159, 2484-2491.

Lee, H.S., Kim, S.K., Han, J.B., Choi, H.M., Park, J.H., Kim, E.C., Choi, M.S., An, H.J., Um, J.Y., Kim, H.M. and Min, B.I. (2006) Inhibitory effects of Rumex japonicus Houtt. on the development of atopic dermatitis-like skin lesions in NC/Nga mice. Br J Dermatol. 155, 33-38.

Leung, D.Y., Boguniewicz, M., Howell, M.D., Nomura, I. and Hamid, Q.A. (2004) New insights into atopic dermatitis. J Clin Invest. 113, 651-657.

Man, M.Q., Hatano, Y., Lee, S.H., Man, M., Chang, S., Feingold, K.R., Leung, D.Y., Holleran, W., Uchida, Y. and Elias, P.M. (2008) Characterization of a hapten-induced, murine model with multiple features of atopic dermatitis: structural,

immunologic, and biochemical changes following single versus multiple oxazolone challenges. J Invest Dermatol. 128, 79-86.
Marsella, R. and Olivry, T. (2003) Animal models of atopic dermatitis. Clin Dermatol. 21, 122-133.
Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W. and Ra, C. (1997) Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. Int Immunol. 9, 461-466.
Navi, D., Saegusa, J. and Liu, F.T. (2007) Mast cells and immunological skin diseases. Clin Rev Allergy Immunol. 33, 144-155.
Nomura, I., Goleva, E., Howell, M.D., Hamid, Q.A., Ong, P.Y., Hall, C.F., Darst, M.A., Gao, B., Boguniewicz, M., Travers, J.B. and Leung, D.Y. (2003) Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol. 171, 3262-3269.
Novak, N. (2009) New insights into the mechanism and management of allergic diseases: atopic dermatitis. Allergy. 64, 265-275.
Novak, N. and Bieber, T. (2005) The role of dendritic cell subtypes in the pathophysiology of atopic dermatitis. J Am Acad Dermatol. 53, S171-176.
Opal, S.M. and DePalo, V.A. (2000) Anti-inflammatory cytokines. Chest. 117, 1162-1172.
Shiohara, T., Hayakawa, J. and Mizukawa, Y. (2004) Animal models for atopic dermatitis: are they relevant to human disease? J Dermatol Sci. 36, 1-9.
Spergel, J.M., Mizoguchi, E., Brewer, J.P., Martin, T.R., Bhan, A.K. and Geha, R.S. (1998) Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest. 101, 1614-1622.
Sugiura, K., Shamoto, M., Sakamoto, N., Shinzato, M., Osada, A., Sugiura, M., Hayakawa, R. and Kato, Y. (2003) It is true that, when Langerhans cells migrate from the skin to the lymph node, they are transported via lymph vessels. Dermatology. 206, 222-224.
Sunada, Y., Nakamura, S. and Kamei, C. (2008) Effect of Lactobacillus acidophilus strain L-55 on the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol. 8, 1761-1766.
Tanaka, T., Tsutsui, H., Yoshimoto, T., Kotani, M., Matsumoto, M., Fujita, A., Wang, W., Higa, S., Koshimoto, T., Nakanishi, K. and Suemura, M. (2001) Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga. Int Arch Allergy Immunol. 125, 236-240.
Taniguchi, Y., Kohno, K., Inoue, S., Koya-Miyata, S., Okamoto, I., Arai, N., Iwaki, K., Ikeda, M. and Kurimoto, M. (2003) Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int Immunopharmacol. 3, 1313-1324.
Theiner, G., Gessner, A. and Lutz, M.B. (2006) The mast cell mediator PGD2 suppresses IL-12 release by dendritic cells leading to Th2 polarized immune responses in vivo. Immunobiology. 211, 463-472.
Wollenberg, A. and Klein, E. (2007) Current aspects of innate and adaptive immunity in atopic dermatitis. Clin Rev Allergy Immunol. 33, 35-44.
Yamanaka, K., Tanaka, M., Tsutsui, H., Kupper, T.S., Asahi, K., Okamura, H., Nakanishi, K., Suzuki, M., Kayagaki, N., Black, R.A., Miller, D.K., Nakashima, K., Shimizu, M. and Mizutani, H. (2000) Skin-specific caspase-1-transgenic mice show cutaneous apoptosis and pre-endotoxin shock condition with a high serum level of IL-18. J Immunol. 165, 997-1003.
Yatsuzuka, R., Inoue, T., Jiang, S., Nakano, Y. and Kamei, C. (2007) Development of new atopic dermatitis models characterized by not only itching but also inflammatory skin in mice. Eur J Pharmacol. 565, 225-231.