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Letters to the Editor

Immunohistological comparison of cutaneous pathology of three representative murine atopic dermatitis models

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disorder characterized by cutaneous injuries, epidermal barrier damages and severe itching [1]. People suffering from AD have eczematous, crusty skin and become vulnerable to infection such as *Staphylococcus aureus* and to other noxious stimuli. Although the development of AD has been previously explained by Type I hypersensitivity, epidermal proliferation and barrier damages are also commonly observed in AD patients. Recently, the importance of the disruption of epidermal barrier function in the development of AD has been demonstrated [2] and the genetic studies have revealed that the mutation or alteration of epidermal barrier proteins like filaggrin (FLG) and loricrin (LOR) might be involved in the genetic predisposition to AD, drawing a tremendous interest in the roles of these proteins in AD.

To investigate AD and evaluate anti-AD effects of candidate drugs, diverse murine models have been developed including spontaneous NC/Nga mice model [3] and induction models using house dust mite extracts or haptens. Of these, epicutaneous AD inducers like *Dermatophagoides farinae* (DF) [4] or haptens like oxazolone (Ox) [5] and trinitrochlorobenzene (TNCB) [6] are frequently used since they can ensure reliable development of AD-like dermatitis in relatively short experimental periods. However, there are few reports which compared these murine models in the aspect of histology, particularly on the distribution or changes in epidermal barrier biomarkers such as FLG or LOR, despite the importance of this information for the selection of a relevant model. In this study, therefore, we compared the histopathology of three representative murine AD models, DF and TNCB-induced NC/Nga mice models and Ox-induced hairless mice model through the immunohistochemistry of epidermis and dermis of AD-like skin lesion along with a routine examination of serum IgE and clinical scoring for disease severity in an effort to provide important information for the future studies on skin barrier disruption associated with AD.

Male NC/Nga and female hairless mice aged 7 weeks old were used for all experiments. According to the methods described previously [4–7], AD-like dermatitis was established by 2-week topical application of DF extract ointment (Biostir AD, Biostir Inc., Hiroshima, Japan) as epicutaneous inducer or TNCB (2-chloro-1,3,5-trinitrobenzene, Fluka Analytical, Germany) for 6 weeks as a hapten in NC/Nga mice, respectively. Meanwhile, AD-like dermatitis in hairless mice was induced by 17-day topical application of Ox (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one, Sigma-Aldrich, St. Louis, MO). Naïve NC/Nga mice were served as a

control for DF and TNCB-model while ethanol (vehicle for Ox)-treated hairless mice was used as a control for OX-model.

Clinically, AD mice exhibited dry, rough skin with multifocal scratching scars on the back (Fig. 1A) and macroscopic examination of erythema, scarring, edema, and excoriation on sensitized dorsal skin at the time of sacrifice revealed that DF-model had the severest total scores regarding every clinical index defined (Fig. 2A) [8]. Histological examination demonstrated that in all three models, AD-like skin lesions were well established including diffuse epidermal hyperplasia, elongation of rete ridges, formation of crusty scales in the epidermis and inflammatory response with lymphocytes, eosinophils in the papillary dermis compared to control groups (Fig. 1B). In addition, prominent T lymphocytic infiltration into the dermoepidermal junction was observed (Fig. 1C). TNCB and OX-models displayed more diffuse infiltration than DF-model. Toluidine blue staining for mast cells showed that degranulated mast cells were increased in dermis (Figs. 1D and 2B) and eosinophils also increased consistently with significantly elevated serum IgE level (Fig. 2C). Meanwhile, several remarkable histological differences (Fig. 2D), including epidermal hyperplasia, lymphocytic infiltration, parakeratosis, and dermal inflammation suggested that NC/Nga mice might be more acceptable for the study of epidermis related pathophysiology than hairless mice while hapten-induced models might be a better choice for examining T cell-mediated hypersensitivity reaction. Increased trans-epithelial water loss (TEWL) and skin thickness, the markers for the disrupted barrier function and epidermal inflammation could be measured only in OX-model owing to the absence of interfering hair coat (Fig. 2E).

FLG combines cytoskeleton and contributes to the transformation of keratinocytes into outermost squamous corneal cells [9]. LOR is a major ingredient of cornified cell envelope, playing a key role in promoting epidermal cornification [9]. Null or mutational changes of either FLG or LOR in human contribute to the development of AD-like skin lesion [10]. To explore the expression of FLG and LOR in three AD models, paraffin embedded tissues from each model were incubated using polyclonal rabbit anti-FLG antibody (ab24584, abcam, Cambridge, UK) or polyclonal rabbit anti-LOR antibody (ab24722, abcam) respectively as a primary antibody overnight and then incubated with anti rabbit HRP-conjugated antibody (sc-2012, Santa Cruz Biotechnology, Inc., CA) for 2 h. Immunoreactivity of FLG confined in the junction between stratum corneum and stratum granulosum in normal animals, extended through the thickened stratum granulosum to suprapinosum layer in AD lesions of all three models (Fig. 1E). Meanwhile, LOR mainly expressed at stratum corneum in normal animals, progressed into whole layer of stratum granulosum but the region of expression was limited within thickened stratum granulosum (Fig. 1F). Control animals in OX-model presented slightly expanded FLG or LOR expression within stratum granulosum, probably due to irritation from ethanol (vehicle for Ox).

* All animal procedures were approved by the AmorePacific Institutional Animal Care and Use Committee (IACUC).

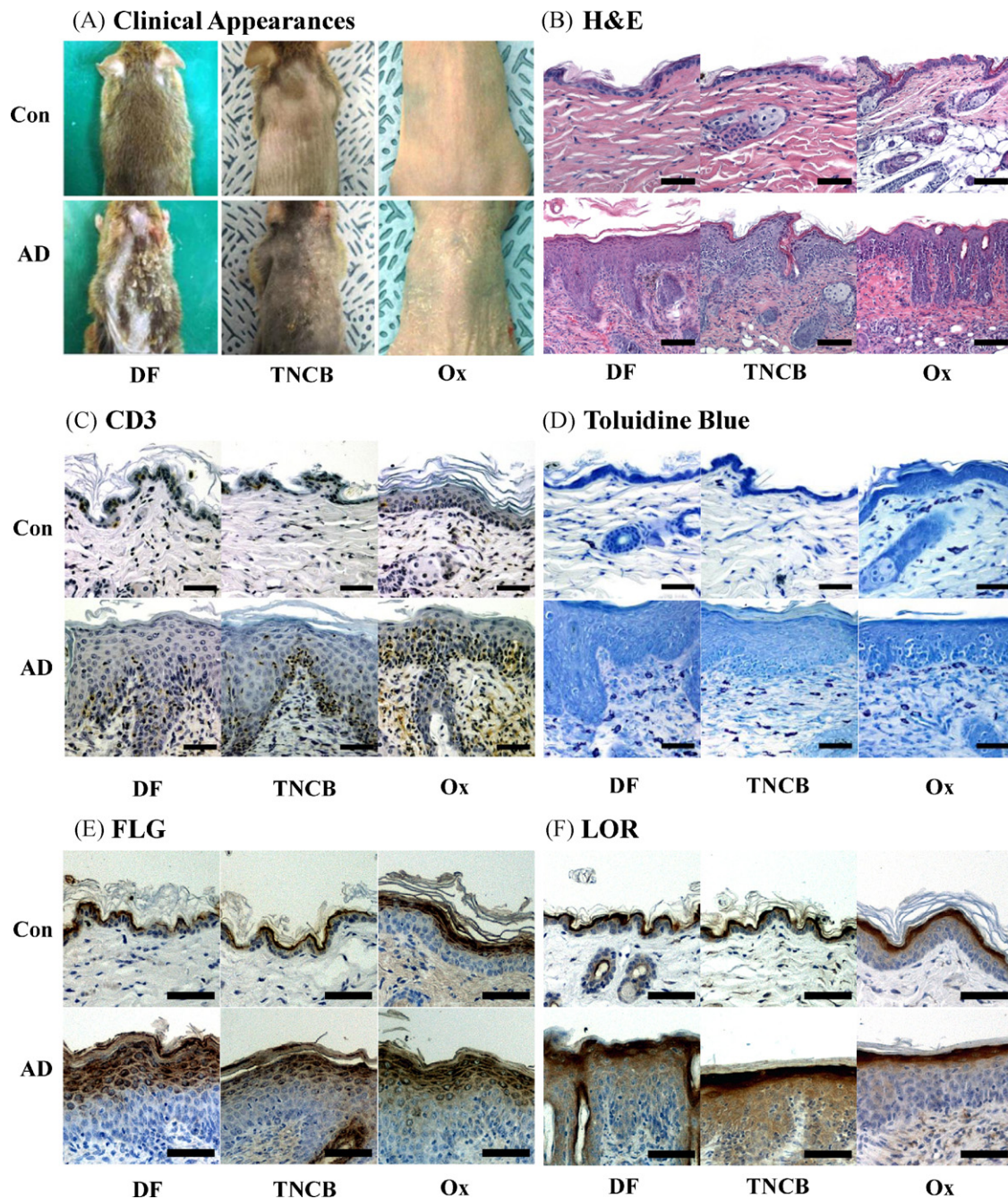


Fig. 1. Histological and immunohistochemical comparison of three murine atopic dermatitis models. (A) Clinical appearances of three AD murine models. Representative photographs of 8–9 animals (B) H&E staining. Sensitized epidermis was significantly thickened and prominent inflammatory response was provoked in the dermis. Bar = 50 μ m. (C) Anti-CD3 staining for the examination of T lymphocytes infiltration. Bar = 50 μ m. (D) Toluidine blue staining for the examination of mast cell distribution and degranulation. Bar = 50 μ m. (E) Anti-FLG staining. Bar = 50 μ m. (F) Anti-LOR staining. Bar = 50 μ m.

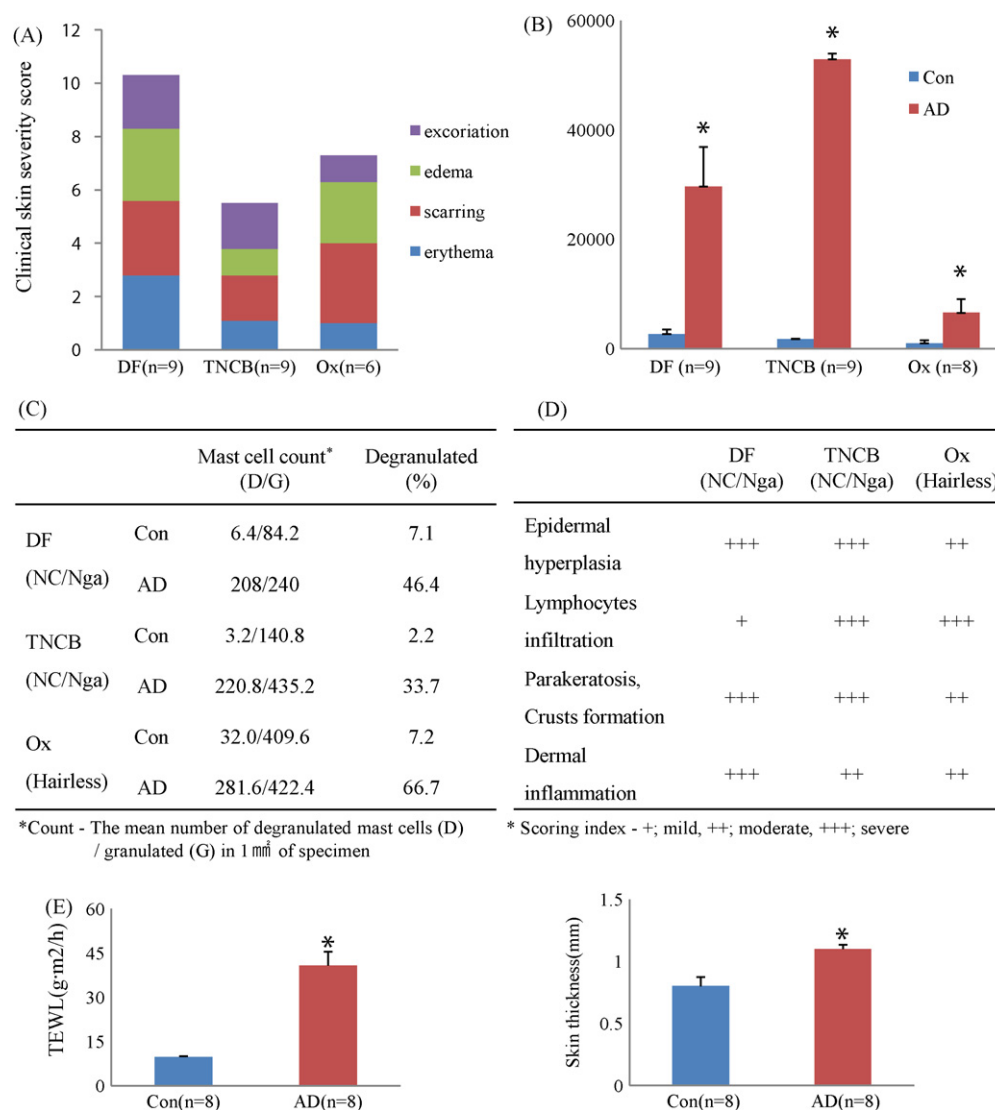


Fig. 2. Morphological, biochemical and functional alteration in three murine atopic dermatitis models. (A) Clinical severity scores were graded from scales 0–4 (0, not present; 1, minimal; 2, slight; 3, moderate; 4, marked) at 24 h (DF-model), 1 week (TNCB-model), or 24 h (OX-model) after the last challenge. (B) Serum IgE levels. Values are presented in ng/mL as mean $\pm$ SD of $N = 8-9$. *Significant difference from corresponding control ($p < 0.05$). (C) The mean number of granulated and degranulated mast cells in 1 mm² area was quantified. (D) Histopathological disease severity scoring. (E) Skin thickness and TEWL measured in OX-model. Values are presented as mean $\pm$ SD of $N = 8-9$. *Significant difference from corresponding control ($p < 0.05$).

In conclusion, it is suggested that NC/Nga mice models might be better choice than hairless mice to study epidermal pathology such as acanthosis whereas hapten-induced models might be superior to DF-model in comprehending T cell-mediated hypersensitivity reaction in AD. Furthermore, it is confirmed that FLG and LOR are aberrantly expressed in the epidermis of all three murine AD models.

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References

- [1] Leung DY, Bieber T. Atopic dermatitis. *Lancet* 2003;361(9352):151–60.
- [2] Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38(4):441–6.
- [3] Matsuda H, Watanabe N, Geba GP, Sperl J, Tsudzuki M, Hiroi J, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997;9(3):461–6.
- [4] Gao XK, Nakamura N, Fuseda K, Tanaka H, Inagaki N, Nagai H. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. *Biol Pharm Bull* 2004;27(9):1376–81.
- [5] Man MQ, Hatano Y, Lee SH, Man M, Chang S, Feingold KR, et al. 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* 2008;128(1):79–86.
- [6] Yamashita H, Makino T, Mizukami H, Nose M. Pharmacological characterization of a chronic pruritus model induced by multiple application of 2,4,6-trinitrochlorobenzene in NC mice. *Eur J Pharmacol* 2007;563(1–3):233–9.
- [7] Lee JH, Jung KM, Bae IH, Cho S, Seo DB, Lee SJ, et al. Anti-inflammatory and barrier protecting effect of lithospermum erythrorhizon extracts in chronic oxazolone-induced murine atopic dermatitis. *J Dermatol Sci* 2009;56(1):64–6.
- [8] Yamamoto M, Haruna T, Yasui K, Takahashi H, Iduhara M, Takaki S, et al. A novel atopic dermatitis model induced by topical application with *Dermatophagoides farinae* extract in NC/Nga mice. *Allergol Int* 2007;56(2):139–48.
- [9] Steinert PM, Marekov LN. The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. *J Biol Chem* 1995;270(30):17702–11.
- [10] Scharschmidt TC, Man MQ, Hatano Y, Crumrine D, Gunathilake R, Sundberg JP, et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009;124(3):496–506, 506.e1–6.

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