Erdr1 Attenuates Dermatophagoides farina Body Extract-Induced Atopic Dermatitis in NC/Nga Mice


TO THE EDITOR

Atopic dermatitis (AD) is a multifactorial inflammatory skin disease characterized by a defective skin barrier and dysregulation of the immune system in terms of Th helper type 1 (Th1) and Th2 cell imbalance (Peng and Novak, 2015). Th2 cytokines, such as IL-4, IL-13, and thymic stromal lymphopoietin, disrupt skin barrier and immune system function and induce inflammation in the lesional skin. Thymic stromal lymphopoietin is significantly increased and activates dendritic cells to produce Th2 chemokine attractants, including chemokine (C-C motif) ligand 17 (CCL17) and CCL22, resulting in an increased Th2-skewed inflammatory responses in the lesional skin (Soumelis et al., 2002).

Previously, erythroid differentiation regulator 1 (Erdr1) was shown to have a negative correlation with IL-18, which is closely associated with AD severity (Kim et al., 2016a; Trzeciak et al., 2011). Erdr1 is first discovered in WEHI-3 cell line and released under stress conditions, modulating growth and survival of cells (Dormer et al., 2004a, 2004b). Although functions and mechanisms of action remain unclear, recent studies show that Erdr1 displays anti-inflammatory and anticancer properties on various skin diseases, including psoriasis, rosacea, and melanoma (Jung et al., 2011; Kim et al., 2015, 2016a, 2016b). Therefore, we hypothesized that Erdr1 would exert protective effects on AD through its anti-inflammatory functions, similar to its effects on psoriasis and rosacea.

Here, we compared the expression level of Erdr1 between AD lesional skin and normal skin from humans and mice. To determine Erdr1 expression in AD lesional skin from human subjects, skin tissues were collected from normal control (n = 5) and patients with AD (n = 11). This experiment using human subjects was approved by the ethical committee of the Catholic University of Korea, and all human subjects provided written informed consent. Also, all experimental procedures with mice were approved by the Institutional Animal Care and Use Committee of Sookmyung Women’s University (SM-IAUC-2013-0726-022). As shown in Supplementary Figure S1a and b online, Erdr1 expression was significantly lower in AD lesional skin than normal skin, and effective treatments restored expression, indicating that Erdr1 may play a critical role in AD. To investigate the role of Erdr1 in AD, Dermatophagoides farina body ointment was topically applied to the ears of NC/Nga mice to induce AD-like skin inflammation. Then, 10 μg/kg of recombinant Erdr1 (rErdr1) or 2 mg/kg of dexamethasone as a positive control was administered by intraperitoneal injection. As shown in Figure 1a, rErdr1 significantly improved the external symptoms of AD-like skin inflammation compared with the group injected with the vehicle, phosphate buffered saline. Histological analysis also showed that rErdr1 administration resulted in decreased inflammatory cell infiltration, such as eosinophils and mast cells, and hyperproliferation in the epidermis.

To determine the severity of AD, symptoms of redness, edema, scaling, and excoriation were scored, and the overall score was evaluated by the sum of each category. The scores were

Abbreviations: AD, atopic dermatitis; CCL17, chemokine (C-C motif) ligand 17; CCL22, chemokine (C-C motif) ligand 22; Erdr1, erythroid differentiation regulator 1; rErdr1, recombinant Erdr1; Th, T helper cell.
Figure 1. Erythroid differentiation regulator 1 (Erdr1) alleviated the characteristic clinical symptoms of atopic dermatitis (AD). (a) Representative lesions on the ear and the back of the head from each group (N = 6/group) are shown. The sections from mouse ear tissues were stained with hematoxylin and eosin to examine histopathological features of the skin lesions 21 days after AD induction. Black arrows indicate the infiltration of eosinophils. Toluidine blue staining was used to determine the infiltration of mast cells in the lesional skin. Red arrows indicate mast cells (scale bar = 60 μm). (b) The severity of AD symptoms was evaluated based on a three-point scoring system. The AD score was graded as 0 (absent), 1 (mild), 2 (moderate), or 3 (severe) based on the sum of the scores of clinical symptoms, such as redness, edema, scaling, and excoriation. (c, d) Ear thickness was measured with a caliper twice a week. On day 21 after AD induction, a 0.8-cm-diameter punch hole was excised from both ears of each mouse (total 12 tissues) and then weighed by using an electronic scale. (e) On day 21, sera were collected, and the concentration of IgE was determined by an ELISA. (f) IL-4 mRNA levels were determined using reverse transcriptase (RT)-PCR. To quantify the intensity of bands for IL-4 expression, the densitometry program, TotalLab100, was used. Densitometry data show the ratio of nucleic acid intensity to the loading control. All values were normalized relative to the normal. Data show the average of IL-4 mRNA expression from three representatives of each group (N = 6/group). To determine systemic IL-4 production, spleen was isolated from each mouse. Spleen size and percentage of CD3⁺CD4⁺ T cells within an equal number of splenocytes were assessed as shown in Supplementary Figure S4a and b online. Treatment with rErdr1 or Dex suppressed enlargement of the spleen; however, there was no difference in T cell percentage between the groups. An equal number of cells (5 × 10⁶) from each group were used to determine cytokine levels. The concentration of IL-4 in splenocyte-conditioned media was measured by an ELISA. Each bar represents the mean ± standard deviation (*P < 0.05, **P < 0.01, ***P < 0.001). A representative experiment of three independent experiments is shown.
considerably decreased by rErdr1 administration compared with those in the vehicle-injected group (Figure 1b and Supplementary Figure S2 online). In addition, ear thickness and ear weight indicative of edema were also examined. rErdr1 treatment markedly reduced both ear thickness and weight, showing decreased hyperproliferation in the epidermis and edema in the ear skin (Figure 1c and d). The effects of rErdr1 on AD were
Further confirmed using 2,4-dinitrochlorobenzene-induced AD in BABL/C mice. rErdr1 alleviated epidermis thickness and mast cell infiltration in 2,4-dinitrochlorobenzene-induced AD lesional skin (Supplementary Figure S3a and b online).

Although AD pathogenesis remains unclear, general characteristics of AD include Th2 polarization and high levels of serum IgE (Peng and Novak, 2015). To determine whether rErdr1 administration could suppress IgE and IL-4 production in the AD mouse model, levels of IgE and IL-4 were measured. Serum IgE levels were significantly increased in mice induced to have AD that were injected with vehicle (Figure 1e). Administration of rErdr1 considerably reduced the serum IgE level in the AD-induced mice, and the reduction range was similar to the dexamethasone-injected group, showing that this dose of Erdr1 has similar preventive potential to dexamethasone. Expression of IL-4 both in the lesional skin and splenocytes also increased on AD induction, and this increase in expression was significantly smaller in the rErdr1-injected group, implying that the distribution of Th2 cells was reduced in rErdr1-injected mice (Figure 1f).

During AD pathogenesis, Th2 chemotaxtractants, CCL17 and CCL22, are increased to recruit the Th2 cells to AD lesional skin (Gros et al., 2009). As shown in Figure 2a, Tslp mRNA expression in AD lesional skin was significantly decreased in the rErdr1-injected group compared with the vehicle-injected group. In accordance with the decreased level of Tslp, both the mRNA and protein levels of CCL17 and CCL22 were also downregulated by rErdr1 injection in the AD lesional skin. Keratinocytes and activated dendritic cells are known to be the main producers of CCL17 and CCL22, respectively, in AD (Soumelis et al., 2002; Vulcano et al., 2001). In the vehicle-injected group, CCL17 and CCL22 were expressed primarily in the epidermis and dermis, respectively, and expression was decreased by rErdr1 injection (Figure 2b).

Next, we determined the effect of Erdr1 on angiogenesis. It has been reported that a representative angiogenic factor vascular endothelial growth factor expression is significantly higher in the stratum corneum of AD than normal stratum corneum, suggesting that keratinocytes in AD lesional skin produce greater amounts of vascular endothelial growth factor. Also, vascular endothelial growth factor levels are highly correlated with AD scores, especially redness and edema (Amarbayasgalan et al., 2012). Figure 2c shows that expression of vascular endothelial growth factor in the lesional skin, especially keratinocytes in the epidermis, was significantly inhibited by rErdr1 injection. CD31 immunofluorescence staining data indicated that angiogenesis was induced in the vehicle-injected group but was significantly decreased by rErdr1 injection (Figure 2d).

This study suggests that rErdr1 has preventive effects on AD. Th2 chemotaxtractants and IL-4 expression were decreased by rErdr1 in an AD-induced mouse model. Erdr1 was previously reported to also have a therapeutic effect on psoriasis, which is a Th1-dominant skin disease (Kim et al., 2016b). These studies imply that rErdr1 might regulate both Th1 and Th2 responses. Recent studies have shown that several therapeutic agents improve the symptoms of AD by increasing generation of Treg. The treatment significantly increases the number of Treg cells, leading to suppression of Th1 and Th2 responses along with reduction of serum IgE in AD (Haitz and Anandasabapathy, 2015; Han et al., 2015; Li et al., 2016). Therefore, further investigation is needed to determine whether Erdr1 suppresses both Th1 and Th2 responses by enhancing generation of Treg.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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MyD88 Contributes to Staphylococcal Enterotoxin B-Triggered Atopic Dermatitis-Like Skin Inflammation in Mice

TO THE EDITOR
Atopic dermatitis (AD) is a chronic pruritic skin inflammation, caused by skin barrier defects and by a dysregulated immune system (Bieber, 2012). Patients with AD often suffer from infections with Staphylococcus aureus, mostly superantigen-producing strains (Boguniewicz and Leung, 2011). Superantigens, including staphylococcal enterotoxin B (SEB), directly crosslink major histocompatibility complex class II on professional antigen-presenting cells with the T-cell receptor to induce T-cell activation independent of antigens (Spertini et al., 1991). SEB-activated human dendritic cells enhance the T helper type 2 (Th2) response in AD (Mandron et al., 2006) and induce homing of T cells to the skin (Strickland et al., 1999). Furthermore, superantigens act as antigens, as patients with AD possess superantigen-specific immunoglobulins (Kotzin et al., 1993). Epicutaneous sensitization of mice with SEB induces skin inflammation, Th2 polarization, and SEB-specific humoral responses (Laouini et al., 2003) and was shown to mediate the itching response via IL-31 (Cevikbas et al., 2014). The combined application of SEB with other allergens to skin or lung enhanced the allergic response to the antigen (Forbes-Blom et al., 2012; Huvenne et al., 2010; Savinko et al., 2005).

Previously, we showed that myeloid differentiation primary response gene 88 (MyD88)-deficient mice display reduced inflammation in a model of ovalbumin (OVA)-driven AD-like inflammation (Didovic et al., 2016). Here, we analyzed the contribution of MyD88 signaling to SEB- and OVA+SEB-induced allergic sensitization in mice. All animal experiments were performed with permission of the government of North Rhine-Westphalia, Germany. Epidermal thickness was higher after SEB treatment compared with solvent controls and strongly enhanced after OVA+SEB treatment in wild-type (WT) mice but barely in MyD88-deficient mice (Figure 1a, Supplementary Figure S1a online). Skin draining lymph node (LN) cell numbers were particularly high after OVA treatment of WT mice, but not further enhanced after OVA+SEB treatment, whereas cell numbers of MyD88-deficient mice were lower (Figure 1b). Dermal F4/80⁺ macrophage numbers were slightly enhanced after SEB or OVA treatment, and even more on combined OVA+SEB treatment. This enhancement was partly dependent on MyD88 (Figure 1c, Supplementary Figure S1c). Of note, dermal Th cell numbers were higher in MyD88-deficient mice than in WT littermates. The highest numbers of dermal Th cells, however, were detected in WT mice after combined OVA+SEB treatment, whereas numbers in MyD88-deficient mice remained largely unchanged compared with solvent control (Figure 1d, Supplementary Figure S1d). Numbers of dermal CDB⁺ T cells as well as monocytes and dermal dendritic cells were only marginally altered (data not shown). Epidermal Langerhans cell numbers were significantly reduced in WT mice after OVA and after SEB treatment compared with solvent controls, and also compared with MyD88-deficient mice. Intriguingly, after combined OVA+SEB application, numbers of epidermal Langerhans cells of WT mice were higher than on treatment with one of the antigens alone and almost as high as in MyD88-deficient mice (Figure 1e, Supplementary Figure S1b), indicating impaired emigration compared with treatment with either antigen alone. Combined OVA+SEB exposure, but not OVA or SEB alone, also led to epidermal T-cell accumulation, which probably were CDB⁺ T cells (Savinko et al., 2005) (Figure 1f–h, Supplementary Figure S1b). Furthermore, a large proportion of epidermal T cells were T-cell receptor beta⁺ T cells, indicating that MyD88 mediates the accumulation of these T cells in the epidermis after OVA+SEB treatment. Thus, the combined application of OVA and SEB

Abbreviations: AD, atopic dermatitis; LN, lymph node; MyD88, myeloid differentiation primary response gene 88; OVA, ovalbumin; SEB, staphylococcal enterotoxin B; Th, T helper cell; WT, wild-type