Extracellular superoxide dismutase ameliorates house dust mite-induced atopic dermatitis-like skin inflammation and inhibits mast cell activation in mice

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Abstract: Extracellular superoxide dismutase (EC-SOD) is an enzyme that catalyses the dismutation of superoxide anions. It has multiple functions, such as reactive oxygen species scavenging, anti-angiogenic, anti-inflammatory, antichemotactic and antitumor activities. Recently, we demonstrated that EC-SOD inhibits ovalbumin-induced allergic airway inflammation in mice. However, the anti-allergic effect of EC-SOD on skin tissue and the role of EC-SOD in mast cells, which are important for allergic responses, have not been well studied. In this study, we investigated whether EC-SOD can alleviate atopic dermatitis in mice and inhibit mast cell activation. Treatment with human recombinant EC-SOD ameliorated house dust mite-induced atopic dermatitis in mice. Furthermore, the levels of pro-allergic cytokine gene expression and histamine release increased in EC-SOD KO mast cells and decreased in EC-SOD overexpressing mast cells, suggesting that EC-SOD inhibits mast cell activation. Consistently, a passive cutaneous anaphylaxis experiment showed more blood leakage from EC-SOD KO mouse ear skin, implying that the lack of EC-SOD increases allergic responses. These results suggest that EC-SOD inhibits mast cell activation and atopic dermatitis and that the loss of EC-SOD causes more severe allergic responses, implying that EC-SOD might be a good drug candidate for treatment of allergic disorders, such as atopic dermatitis.

Abbreviations: AD, atopic dermatitis; BMMC, bone marrow-derived mast cell; DNP-IgE, dinitrophenol immunoglobulin E; DC, dendritic cells; EC-SOD, extracellular superoxide dismutase; ELISA, enzyme-linked immunosorbent assay; HDM, house dust mite; HSA, human serum albumin; KO, knockout; PCA, Passive cutaneous anaphylaxis; PMA, phorbol myristate acetate; TG, transgenic; TSLP, thymic stromal lymphopoietin.

Key words: atopic dermatitis – extracellular superoxide dismutase – mast cells – NC/Nga mouse

Accepted for publication 4 April 2016

Introduction
Atopic dermatitis (AD) is a common chronic skin inflammatory disease. The pathogenesis of AD might result from the complicated interaction between environmental agents and the immune system (1). It is known that an imbalance of CD4+ T cell subsets, in which Th2 differentiation of naïve CD4+ T cells is predominant, causes AD. Indeed, AD is characterized by increased Th2 cells in the spleen and lymph nodes, upregulated expression of Th2 cytokines, such as IL-4, IL-5 and IL-13, and upregulation of thymic stromal lymphopoietin (TSLP) in keratinocytes (2). Recent studies have suggested that Th17 cells are also implicated not only in autoimmune diseases but also in allergic disorders (3,4). However, more experiments are necessary to elucidate how IL-17 is involved in allergic responses.

Chemokines are also implicated in the pathogenesis of AD. Chemokines can attract leucocytes into the tissue where they are overexpressed. Clinical and experimental evidences indicate that expression of several chemokines, such as CCL11, CCL17 and CCL22, is enhanced in AD patient skin lesions. Thus, overexpression of chemokines may play a crucial role in the development and maintenance of AD (5,6).

Another important characteristic is the elevated level of IgE found in AD patient serum. Th2 cytokines, IL-4 and IL-13, induce isotype switching to IgE production. IgE binds to its receptor in mast cells and results in mast cell activation after allergens bind to the IgE. Therefore, Th2 cells are implicated in allergic diseases (7), and inhibition of Th2 cell development and suppression of Th2 cytokine expression are major target processes that regulate allergic responses along with inhibition of mast cell activation, which is also critical in the induction of allergic responses.

Mast cells are derived from hematopoietic progenitor cells and matured in local tissues such as skin (8). They are implicated in allergic diseases, such as AD, asthma and rhinitis. Activation of mast cell induces the release of many pro-inflammatory mediators, such as IL-4, IL-5, IL-6 and TNF-α, and arachidonic acid metabolites, such as leukotrienes and prostaglandins from the granules of mast cells (9). Thus, controlling mast cell activation is critical in the treatment or prevention of allergic diseases.

Extracellular superoxide dismutase (EC-SOD), which is also known SOD3, is an enzyme that catalyses the dismutation of superoxide anions. Although the main function of EC-SOD is in reactive oxygen species (ROS) scavenging, it has many other functions, such as anti-angiogenic, anti-inflammatory, antichemotactic and antitumor activities (10–13). Recently, we reported that EC-SOD could inhibit ovalbumin (OVA)-induced allergic airway inflammation, suggesting that EC-SOD has an anti-allergic effect.
serum IgE level is decreased; (iii) expression of cytokines and chemokine involved in the pathogenesis of AD is decreased and (iv) the percentage of Th2 cells in the spleen and lymph node is reduced. These results suggest that EC-SOD has an anti-allergic effect and may inhibit AD-like skin inflammation in mice.

The anti-allergic effect of EC-SOD might be through inactivation of dendritic cells (DC), inhibition of Th2 cell differentiation or suppression of mast cell activation. It was previously reported that EC-SOD could inhibit DC activation (20). It is also reported that EC-SOD could inhibit Th2 cell differentiation in vitro (14) and pro-inflammatory response in keratinocytes (10). In this study, we showed that EC-SOD could inhibit mast cell activation. However, a previous report demonstrated that mast cells are not important for the development of Th2-mediated skin inflammation (31). Thus, it might be possible that suppression of AD-like skin inflammation by EC-SOD might mainly result from combined inhibitory effects of EC-SOD on DC activation, pro-inflammatory responses of keratinocytes and Th2 cell differentiation.

In this study, we demonstrated that treatment with EC-SOD inhibits HDN-induced AD-like skin inflammation in NC/Nga mice. These findings give us new insights into the treatment of AD and imply that EC-SOD could be a good candidate for the treatment of allergic diseases such as AD.

Author Contributions
YSL designed the experiments, performed the experiments, analysed the data and wrote the manuscript; JHC bred and analysed knockout and transgenic mice; JHL, WL and WTK analysed the data; HWL generated transgenic mice and analysed the mice; and TYK designed the experiments, performed the data analysis and wrote the manuscript.

Funding
This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2013M3A9A3050567).

Conflict of interest
The authors have declared no conflicting interests.

Supporting Information
Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. EC-SOD inhibits AD-related cytokine and chemokine expression. Expression level of (a) cytokines and (b) chemokines in HDN-induced NC/Nga mouse skin was determined by real-time PCR. *P < 0.05 and **P < 0.005.

Figure S2. EC-SOD inhibits IL-17 expression in activated BMMC. BMMCs from WT, EC-SOD TG and EC-SOD KO mice were activated with UNP-IgE and DNP-HSA. Relative expression levels of IL-17 were determined by real-time PCR. A representative example of three independent experiments is shown. *P < 0.05. WT: wild type, TG: EC-SOD transgenic mice, KO: EC-SOD knockout mice.

Figure S3. EC-SOD inhibits cytokine expression and production in BMMC. BMMCs were activated by PMA/A23187, and expression level of IL-6 and TNF-α (a) and production of IL-6 and TNF-α (b) were determined by real-time PCR and ELISA, respectively. *P < 0.05 and **P < 0.005.

References