Fermented Barley Extract Suppresses the Development of Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice, Probably by Inhibiting Inflammatory Cytokines

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We have found that fermented barley extract (FBE), prepared from barley shochu residue, alleviates allergic rhinitis in OVA-sensitized mice. In this study, we examined to determine whether FBE suppresses the development of atopic dermatitis (AD)-like skin lesions in NC/Nga mice. The development of AD-like skin lesions in a 5% FBE containing diet group was significantly inhibited, and scratching behavior, one of aggravating factors, was also suppressed. Neither serum immunoglobulin E (IgE) levels nor interleukin (IL)-4 production by spleen cells in the 5% FBE diet group was found to be significantly reduced. On the other hand, interferon-gamma (IFN-γ) and IL-17 production by spleen cells in the 5% FBE diet group was significantly reduced. Hence it was assumed that FBE alleviates AD-like skin lesions in NC/Nga mice, probably by modulating the cytokine production involved in chronic inflammation, such as IFN-γ or IL-17.

Key words: fermented barley extract; allergy; atopic dermatitis; interferon-γ (IFN-γ); interleukin-17 (IL-17)

Shochu is a Japanese spirit produced from rice, barley, sweet potato, and so on. It generates an abundance of residue after the distillation process, and treatment of the residue is distressing to manufacturers, for the following reasons: Dumping of the residue in the sea is severely restricted by the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter. Besides, application to composts or animal fodders is costly. Recently, shochu residue is expected to serve as food material, since it has been reported to have biological activities, such as prevention of hepatic damage.1,2)

In our laboratory, barley shochu residue is filtered, and the supernatant is evaluated for physiological functions. The supernatant, fermented barley extract (FBE), contains various compounds, such as free amino acids, organic acids, peptides, and polysaccharides. Recently, we reported that FBE alleviates allergic rhinitis in ovalbumin (OVA)-sensitized mice by suppressing differentiation towards Th2 cells.3)

Atopic dermatitis (AD) is a chronic inflammatory skin disease accompanied by severe itching and repeated episodes. Most AD patients have a family history of atopy, and develop dermatitis from infancy, with high serum immunoglobulin E (IgE).4–6) AD patients often suffer from severe itching, which is a serious problem because it not only causes, emotional stress, but also scratching, which is one of the aggravation factors.7)

NC/Nga mice have been reported to develop dermatitis with IgE hyperproduction that is very similar to human AD when they are kept under conventional conditions.8–10) In addition, it has been reported that they develop AD-like skin lesions under repeated application of hapten under SPF conditions.11) Both models are frequently used as a human AD model.12–15)

In the present study, we examined to determine whether FBE suppresses AD-like skin lesions in NC/Nga mice developed by repeated application of hapten. We found that FBE alleviates AD-like skin lesions in NC/Nga mice without suppressing IgE. The production of cytokines (IFN-γ, IL-4, and IL-17) by spleen cells of FBE-fed mice is also discussed.

Materials and Methods

FBE preparation. Barley shochu residue was provided by Yaegaki Sake and Spirits (Hyogo, Japan). Ten liters of Barley shochu residue was centrifuged at 10,000 × g for 20 min. After centrifugation, the supernatant was filtered through no. 2 filter paper (Advantec, Tokyo). The resulting filtrate (6 liters) was freeze-dried and used as fermented barley extract (FBE) in animal experiments in this study. The composition of FBE was as follows: carbohydrate 27.9%, protein 35.0%, dietary fiber 22.0%, fat 5.3%, ash 3.5%, water 6.3%, and polyphenol 2.4%.

Mice. Female NC/Nga mice (5 weeks old) were obtained from Japan SLC (Hamamatsu, Japan). The mice were housed in an air conditioned room (22 ± 2°C) under a 12 h light/dark cycle (light, 7:00 AM–7:00 PM). They were fed a commercial diet (CE-2, CLEA, Tokyo) and water ad libitum. Experiments were undertaken following the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science. After aclima-

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Abbreviations: AD, atopic dermatitis; FBE, fermented barley extract; IgE, immunoglobulin E; IFN-γ, interferon-γ; IL, interleukin; PiCl, picryl chloride
zation, the mice were divided into three groups (n = 7 each). Each group was given one of three experimental diets based on AIN-93G for 7 weeks. The experimental diets contained FBE at 0%, 2%, or 5% (Table 1). Picryl chloride (PiCl) was given as described below after 1 week from the beginning of the experimental diets.

Atopic dermatitis mice. The abdomen and dorsal skin of the NC/Nga mice were shaved, and the next day the shaved abdomen was sensitized with 150 μl of 5% PiCl dissolved in an ethanol and acetone mixture (4:1). On day 5 after sensitization, the dorsal skin and both ears were challenged with 150 μl of 1% PiCl dissolved in olive oil. Challenge by 1% PiCl was repeated once a week for 6 weeks. The severity of dermatitis was assessed once a week by the following scoring procedure: 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe) for each of the five symptoms (itching, edema, hemorrhage, excoriation/erosion, and scaling/dryness), expressed as the sum of the scores for the five symptoms (full score, 15). The number of acts of scratching was counted for 20 min after 1 h of the 6th PiCl challenge using a digital video camera.

Measurement of serum IgE. Blood was collected from the tail vein at 1, 3, and 6 weeks. Serum samples were obtained by centrifugation and stored at −80 °C until use. Total serum IgE levels were measured using an ELISA kit (Bethyl Laboratories, Montgomery, TX).

Measurement of cytokine production. Mice (n = 5 from each group) were killed by cervical dislocation following deep anesthesia. Their spleens were aseptically removed. Single-cell suspensions were prepared by gently squeezing each spleen between two sterile glass slides, and the red blood cells were removed using Tris-buffered NH4Cl solution. The cells were washed in PBS supplemented with 1% fetal bovine serum (FBS) and then suspended in RPMI 1640 medium supplemented with 10% FBS. The cells (5 × 10⁶ cells/ml) were cultured in a 48-well culture plate with concanavalin A (1 μg/ml) at 37 °C for 24 h in a 5% CO2 incubator. The culture supernatant was collected by centrifugation and stored at −80 °C until assay. The concentrations of IFN-γ, IL-4, and IL-17 were determined with an ELISA kit (eBioscience, San Diego, CA).

Statistical analysis. Data are expressed as means ± SE. Statistical significance was tested by Dunnett’s multiple comparison test following one-way analysis of variance and Student’s t-test for two-sample comparison. P values less than 0.05 were considered statistically significant.

Results

The NC/Nga mice were divided into three groups, and were given the experimental diets for 7 weeks (Table 1). During this period, differences in body weight among the control and FBE (both 2% and 5%) diet groups were not found to be significant (Fig. 1).

As shown in Fig. 2, the mice in the control diet group gradually developed AD-like lesions, depending on the times of PiCl challenge. In contrast, the mice in the 5% FBE diet group showed inhibited development of AD-like lesions, and significant differences in the clinical skin severity score were observed between the control and the 5% FBE diet group after the 4th PiCl challenge (Fig. 2).

The number of acts of scratching was counted for 20 min at the 6th PiCl challenge. In the 5% FBE diet group, the number of acts of scratching was significantly suppressed as compared with the control diet group (Fig. 3) (59.1 ± 7.5 times for the control diet group, 19.4 ± 1.2 times for the 5% FBE diet group).

Serum samples were collected at 1, 3, and 6 weeks, and serum IgE levels were determined by ELISA. As shown in Fig. 4, the serum IgE levels in the control diet group gradually increased. The serum IgE levels in the 5% FBE diet group tended to be lower than those in control diet group at 6 weeks, but there was no significant difference in serum IgE levels between the two groups of mice.

<table>
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<th>Control</th>
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<th>5% FBE</th>
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<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Corn oil</td>
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<td>7</td>
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</tr>
<tr>
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<td>3.5</td>
<td>3.5</td>
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<tr>
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*Fermented barley extract.

Table 1. Compositions of Experimental Diets

Fig. 1. Effects of FBE on Body Weight in NC/Nga Mice. ○, control; ●, 2% FBE; △, 5% FBE. Each point represents the mean ± SE for seven mice.

Fig. 2. Effects of FBE on Development of AD-Like Skin Lesions in NC/Nga Mice in Response to Repeated PiCl Challenge. Clinical skin severity scores (full score, 15) monitored once a week. ○, control; ●, 2% FBE; △, 5% FBE. Each point represents the mean ± SE for seven mice. **Significantly different from the control group at p < 0.05 and p < 0.01 respectively.
To determine the immunoregulatory effects of FBE in the NC/Nga mice, we investigated cytokine production by spleen cells. As shown in Fig. 5, production of IFN-γ and IL-17 was significantly suppressed in the 5% FBE diet group as compared with the control diet group, but no significant change in the production of IL-4 was observed between the two groups of mice.

Discussion

We have reported that FBE alleviates allergic rhinitis in OVA-sensitized mice by suppressing differentiation towards Th2 cells. In this study, we examined to determine whether FBE suppresses AD-like skin lesions, and whether FBE alters serum IgE levels and cytokine status in NC/Nga mice. AD-like skin lesions in NC/Nga mice was developed by repeated application of PiCl. FBE effectively lowered the skin severity score (Fig. 2) and suppressed scratching behavior (Fig. 3). Atopic dermatitis is often accompanied by severe itching and it causes scratching. Hence it is of significance that FBE suppressed not only the development of AD-like skin lesions, but also itching.

Unexpectedly, however, FBE failed to suppress serum IgE elevation (Fig. 4). In addition, IL-4 production from spleen cells was not suppressed (Fig. 5B). It is well known that IgE is necessary for the allergic reaction, and that IgE class switching in B cells is induced by IL-4 produced by Th2 cells. It has been reported that serum IgE levels in NC/Nga mice with AD-like skin symptoms are elevated, similarly to most AD patients. What is the explanation for the suppressing effects of FBE on skin lesions in NC/Nga mice without suppression of IgE?

Recently, Yagi et al. reported that STAT6-deficient NC/Nga mice in the absence of the Th2 response developed AD-like skin lesions similarly to wild-type NC/Nga mice. Hence the Th2-mediated immune re-
sponse might not be necessary for the development of AD-like skin lesions in NC/Nga mice. Thus it is probable that imbalance of Th1/Th2 is not the main cause of the development of skin lesions in NC/Nga mice even under IgE-elevation conditions. Instead, a highly inflammatory status might be crucial to the skin lesion in this model.

Under such conditions, IFN-γ acts as a proinflammatory cytokine. Indeed, Taniguchi et al.\(^1\) reported that suppression of AD-like skin lesions in NC/Nga mice by oral administration of royal jelly was related to down-regulation of IFN-γ production. Lee et al.\(^1\) also reported that *Astragalus membranaceus* might suppress AD-like skin lesions in NC/Nga mice by reducing IFN-γ production. These reports suggest that Th1-type cytokine IFN-γ plays a crucial role in the development of AD-like skin lesions in NC/Nga mice. In addition, current studies on humans suggest that IFN-γ is implicated in the chronicity of AD.\(^1^9,20\)

In this study, IFN-γ production in the 5% FBE diet group was significantly inhibited (Fig. 5A), and hence the suppression of AD-like skin lesions by FBE might involve Th1-cell mediated immune responses, rather than Th2 cells. On the contrary, in our previous study, FBE suppressed differentiation towards Th2 cells in OVA-sensitized Balb/c mice. It is obvious that the effects of FBE are different depending on the mouse strain. Although further research is needed on this point, it is probable that the evaluation in this study occurred in an antigen-independent way in the mice with inflammation, while our previous study was conducted in an antigen (OVA)-dependent way in Th2-skewed mice.

Recently, another subset of T cells (Th17 cells) that produce IL-17 has been identified.\(^21\) IL-17 is a proinflammatory cytokine involved in autoimmunity and allergic responses. Nakae et al.\(^22\) reported that allergic diseases such as contact hypersensitivity, delayed-type hypersensitivity, and airway hypersensitivity were significantly reduced in IL-17-deficient mice, and suggested that IL-17 plays an important role in allergic responses. In this study, it was found that IL-17 production by spleen cells in the 5% FBE diet group was significantly inhibited (Fig. 5C). This might also have been involved in the suppression of AD-like skin lesions.

To date, it remains unclear what compound in FBE is responsible for suppression of IFN-γ and IL-17 production. Very recently, Tanabe et al.\(^23\) reported that bifidobacteria suppressed IL-17 production in mice splenocytes and the colon. Since FBE is prepared by filtration of barley shochu residue, it is thought to contain soluble components of microbial cells used in shochu processing, and those components might be involved in the suppression of inflammatory cytokine production. Further evaluation is needed to identify the responsible compounds, and to determine the mechanism of this activity.

In conclusion, this study suggests that FBE inhibits the development of AD-like skin lesions in NC/Nga mice and modulates the cytokine production involved in chronic inflammation such as IFN-γ and IL-17. Hence it is expected that FBE will prove effective in alleviating atopic dermatitis.

References

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