Effect of fermented okara (bean curd lees) intake on TNCB (2, 4, 6-trinitrochlorobenzene)-induced chronic dermatitis in NC/Nga mice

Toshiki Enomoto\textsuperscript{a}, Masato Nishi\textsuperscript{a}, Florin Barla\textsuperscript{a}, Nana Murata\textsuperscript{a}, Hiroshi Matsui\textsuperscript{b}, Hidehiko Kumaga\textsuperscript{b}, Harumi Take\textsuperscript{c}, Toshihide Michihata\textsuperscript{c}, Shizuo Nakamura\textsuperscript{c}, Masao Kawashima\textsuperscript{d}, Eiji Fujihara\textsuperscript{d}

\textsuperscript{a}Department of Food Science, Ishikawa Prefectural University, Nonoichi, Ishikawa 921-8836, Japan (enomoto@ishikawa-pu.ac.jp)
\textsuperscript{b}Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Nonoichi, Ishikawa 921-8836, Japan (hidekuma@ishikawa-pu.ac.jp)
\textsuperscript{c}Laboratory of Food Processing, Industrial Research Institute of Ishikawa, Kanazawa, Ishikawa 920-8023, Japan (take@irii.jp)
\textsuperscript{d}Habutaetoufu Co., Ltd, Kanazawa, Ishikawa 921-8054, Japan (fujihara@habutae.co.jp)

\textbf{ABSTRACT}

Diets containing 1% and 2% fermented okara were administrated to NC/Nga atopic dermatitis model mice. The mice were sensitized by using a solution of 1% TNCB, on ears and scruff zone weekly for 11 weeks to induce skin lesion atopic dermatitis-like. To evaluate the pathology of allergy the thickness of ears was measured. We examined also clinical skin changes, transepidermal water loss, hair loss and fragments of tissue loss. The symptoms of mice fed a diet containing okara were significantly reduced as compared with control group. Th1/Th2 balance of the okara groups was higher than in control. In addition, IL-4 production was significantly decreased in okara diet groups. These results suggests that fermented okara by \textit{Bacillus coagulans}, have anti-allergic effect, considerably attenuate the symptoms in TNCB-induced chronic dermatitis in NC/Nga mice.

\textbf{Key words:} by-product, okara, lactic acid bacteria, chronic dermatitis, anti-allergy, mice

\textbf{INTRODUCTION}

Atopic dermatitis (AD) is a complex eczematous skin disease accompanied by severe itching and frequently repeated episodes. This disease is often linked with a high level of immunoglobulin E (IgE) antibodies against various allergens. IgE production is regulated by several cytokines. The IgE production induced by IL-4 is inhibited by Th1 type cytokines such as INF-\gamma. The prevalence of AD has increased steadily in recent decades [1]. Anti-allergic functional food has been attracting attention, and lactic acid bacteria have been one of the main focuses of reports [2]. In East Asian countries, tofu is a popular food. Okara is produced as a by-product of tofu manufacturing. Although okara is a rich source of nutrients such as dietary fiber, protein, oligosaccharide, mineral and vitamins, the most of it is disposed and unused [3, 4]. Also, Wang and Murphy (1996) [5] reported that okara contains over 10% isoflavones such
as daidzin, genistin and genistein. Therefore the development of a processing way for utilization is expected.

The aim of the study was to investigate the effect of fermented okara intake on TNCB (2, 4, 6-trinitrochlorobenzene)-induced chronic dermatitis in NC/Nga mice. This kind of mice develops AD-like skin lesions under conventional care or upon treatment by repeated challenge with a mite antigen under the specific pathogen-free condition. The induced dermatitis is accompanied by an elevated serum IgE level, increased expression of Th2 cytokines and frequent scratching behaviour which are characteristic features of human AD.

MATERIALS & METHODS

Freeze-dried fermented okara with *Bacillus coagulans* was prepared and used as a feed. Four weeks-old NC/Nga male mice which have a predisposition to spontaneously develop AD-like dermatitis were purchased from Japan SLC, Inc. Control feed, MF was purchased from Oriental Yeast Co. Ltd. Mice were fed MF for 6 weeks without TNCB treatment in a room maintained at controlled temperature (24±2°C), humidity (60±10%), and lighting (9:00 to 21:00 hours) and housed in individual cages. Water and food were available *ad libitum*. Next, they were randomly divided into three groups (n=6/group). The control group was fed a standard MF and the experimental groups were fed a mixture of the standard MF plus fermented okara to give a final fermented okara concentration of 1% (1% FO) and 2% (2% FO), respectively. Then, mice were sensitized initially, at the beginning of experiment, with about 20 µl of TNCB 5% solution onto ventral zone and feet. The following weeks, the TNCB 1% solution was applied to sensitized ears and scruff zone once per week as previously described by Kitagaki et al., (1995) [6].The animals were fed these diets for 5 weeks and ear thickness was measured with an electronic caliper every week just before sensitization. Also, we examined clinical skin change and scratching score for 60 minutes was evaluated in all groups. The whole blood was collected from inferior vena cava, under anaesthesia with diethyl ether, and placed immediately into ice. Serum samples were obtained by centrifugation, and were used to quantify the level of cytokine (IFN-γ and IL-4). IFN-γ and IL-4 in serum samples were assayed by commercial ELISA kit (RD SYSTEMS). Experimental procedures were approved by the Animal Care and Use Committee of Ishikawa Prefectural University, Ishikawa, Japan.

RESULTS & DISCUSSION

In the present study, TNCB was used as the hapten which was applied to the auricles and scruff zone of NC/Nga mice once a week for 11 weeks continuously. Ear thickness of mice was measured weekly and an increase in the thickness was observed in all groups. However, mice in the fermented okara-administrated groups had a significantly smaller ear thickness compared with the control group (Fig.1). Oral administration of fermented okara to NC/Nga mice inhibited the development of AD-like skin lesions based on the total skin severity scores, while the scores considerably increased in the control group as it is shown in the Fig. 2. All animals treated with fermented okara diets were in good health throughout the experiment and did not show signs of abnormalities or disease. There were no significant differences in body weight gain between the control and okara diets groups. Furthermore, the weights of internal organs collected after ventrotomy did not differ among the three groups (data not shown). These
results suggest that the symptoms of mice fed a diet containing fermented okara were markedly reduced.

**Figure 1.** Effect of okara administration on sensitized ears thickness induced by TNCB application in NC/Nga mice. Results are expressed as the mean ± SD of six mice per group. Means with the different letters are significantly different at the 5% level (Tukey’s multiple range test). ●: control diet, ▲: 1% FO diet, ■: 2% FO diet

**Figure 2.** Effect of okara administration on the total clinical score induced by TNCB application in NC/Nga mice. Results are expressed as the mean ± SD of six mice per group. Means with the different letters are significantly different at the 5% level (Tukey’s multiple range test). ●: control diet, ▲: 1% FO diet, ■: 2% FO diet
The development of dermatitis is accompanied by an increase in serum IgE levels and an increase of IL-4 and decrease of IFN-γ levels. Secretion of a Th1 cytokine (IFN-γ) and a Th2 cytokine (IL-4) were determined by ELISA using a kit regent. Figure 3 shows the IFN-γ level of each group and the Figure 4 shows the level of IL-4. In addition, the IFN-γ level of serum was not affected by fermented okara administration in both mice groups but in contrast the IL-4 level was significantly decreased, indicating that the Th1/Th2 balance of fermented okara groups was higher than the control. Therefore, these results suggested that okara fermented by
Bacillus coagulans suppressed the production of the Th2-type cytokine IL-4 and improve the balance of Th1/Th2 in a model of AD. In maintaining the homeostasis, the Th1/Th2 balance is considered to be essential. T-cells maintain well-balanced relations in the modulation of cytokine secretion to retain homeostasis; disruption of this balance induces various immunologic diseases. Allergen-induced Th1/Th2 imbalance causes inflammation in the allergic response. In such conditions, Th1 cells mediate autoimmune diseases, and abnormal Th2 response is involved in allergic diseases [7, 8]. It is thought that the anti-inflammatory effect of Genistein in Ng/Nga mice is due to suppression of chemical mediators released from mast cell as a key effector cell in early-phase allergic inflammation [9]. Similar effect can be also expected for the isoflavones contained in okara. However, further study on the production of other cytokines by lymphocytes and antigen-presenting cells will enhance our understanding of the anti-allergic activity of okara.

CONCLUSION

The overall results indicate that fermented okara exerts an anti-allergic action through suppression of Th2-type immune response. Thus, okara fermented by Bacillus coagulans might be effective dietary supplement for the prevention of AD.

REFERENCES


