

# Effect of fermented papaya preparation on dermal and intestinal mucosal immunity and allergic inflammations

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## Abstract

**BACKGROUND:** Fermented papaya preparation (FPP) from yeast fermentation of *Carica papaya* Linn is a natural health food that is commercially sold in Japan. A previous study revealed that FPP has antioxidant activity. However, the effect of FPP on allergic diseases remains unclear. The aim of the present work was to examine whether the oral administration of FPP to mice restrained two types of contact hypersensitivity models, FITC (Th2 type) induced ear and colon oedema, and oxazolone (Th1 type) induced ear and colon oedema.

**RESULTS:** The sensitisation of FITC or oxazolone increased the plasma levels of IL-10, IFN- $\gamma$ , and TNF- $\alpha$ . Histological examinations revealed a marked increase of IgA, dendritic cells and inflammatory cells in the colon. When the animals were given FPP before sensitisation by FITC or oxazolone, all the events induced by either FITC or oxazolone decreased markedly.

**CONCLUSION:** These results suggest that the oral administration of the FPP may have a therapeutic potential for the prevention of contact hypersensitive immuno-response.

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**Keywords:** fermented papaya preparation; TNF- $\alpha$ ; FITC; oxazolone

## INTRODUCTION

A variety of food factors, including natural antioxidants, affect mucosal immunity, the autonomic nervous system and the neuro-endocrine system.<sup>1</sup> The perturbation of these systems often increases the generation of reactive oxygen species (ROS) and affects gastrointestinal functions, thereby inducing mucosal allergic reactions and inflammatory bowel diseases.<sup>2</sup> Allergic reactions, such as hay fever and contact dermatitis, are well known to have increased rapidly during the last 50 years and they have now become one of the major issues in Japan, thus suggesting marked changes in the reactivity of mucosal immunity and neuro-immune network.<sup>3</sup> Previous studies by Imao *et al.*<sup>4</sup> revealed that fermented papaya preparation (FPP) decreased ROS generation and effectively inhibited the oxidative stress-induced GSH decrease in neuronal cells, thus protecting them from apoptosis through both antioxidant- and bax/bcl-2-sensitive pathway(s). This suggests that FPP may modulate the mucosal neuro-immune network. To test this

hypothesis, the effect of FPP on the type IV allergic reactions in the skin and intestine was investigated. Th1-dependent type IV and Th2-dependent mucosal allergic reactions were elicited in the skin and intestine of male ICR mice by repeated sensitisation with 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone)<sup>5</sup> and fluorescein isothiocyanate (FITC),<sup>6</sup> respectively. The oral administration of FPP inhibited the allergic reaction elicited by FITC and oxazolone on the skin and mucosa in the large intestine. Based on these observations, the mechanism by which FPP modulated the mucosal immunity and suppressed allergic inflammation in the skin and intestine is discussed.

## MATERIALS AND METHODS

### Mice

Specific pathogen-free male ICR mice were purchased from Japan SLC (Shizuoka, Japan). The mice were 8 weeks old at the beginning of each experiment

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and were housed in filter-protected cages. Ambient light was controlled automatically to produce a 12-h light/12-h dark cycle, and sterile water was provided *ad libitum*. The animals were subjected to experiments according to the animal care regulations of Osaka City University Medical School.

### Chemicals

Oxazolone was obtained from Sigma-Aldrich Chemical (Milwaukee, WI), FITC isomer I was supplied by Dojindo (Kumamoto, Japan). Oxazolone solution was dissolved in 1:1 v/v ethanol: distilled water and FITC solution was dissolved in 1:1 v/v acetone: dibutyl phthalate. Solutions were prepared freshly immediately prior to dosing.

### Contact sensitisation

The skin on the back of each mouse was shaved with electric clippers and used as a sensitising area. The mice were sensitised by applying 50  $\mu$ L of 0.5% FITC or 0.5% oxazolone solution. The mice were challenged for 5 days after sensitisation by applying the FITC or oxazolone solution (4  $\mu$ L) to both the dorsal and ventral surfaces of the right ear. Ear thickness was measured with a micrometer, and CHS was regarded as the difference between the ear thickness measured before challenge and that measured 24 h after challenge. In the colon, either FITC or oxazolone solution was administered through the rectum via a 3.5 F catheter equipped with a 1 mL syringe. The catheter was inserted so that the tip was 4 cm proximal to the anal verge and the oxazolone or FITC was injected with a total volume of 100  $\mu$ L. To ensure distribution of the oxazolone or FITC within the entire colon, mice were held in a vertical position for 30 s after the injection. The colon was removed 24 h after challenge and then the morphological and immunological changes were observed.

### FPP treatment

FPP was obtained from Saido Co. Ltd. (Fukuoka, Japan). It was made by yeast fermentation of *Carica papaya* Linn. The preparation is shown in Fig. 1. Six milligrams per day of FPP in ultra-pure water was administered orally to the mice throughout the experimental period. Only ultra-pure water was administered to the control animals and dextrose (6 mg/day) was administered, because the FPP preparation included dextrose.

### Quantification of cytokines by enzyme-linked immunosorbent assay

Blood samples were taken from the heart 5 h after sensitisation, and then the plasma was fractionated. The plasma interleukin-10 (IL-10), interferon-gamma (IFN- $\gamma$ ) and tumour necrosis factor-alpha (TNF- $\alpha$ ) concentrations were determined using a commercial ELISA kit (Endogen, Rockford, IL) according to the manufacturer's instructions. The optical density

was measured with a microplate reader (Molecular Devices, Sunnyvale, CA).

### Histological analysis

The colon specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue Tek, OCT compound, and cut into 5  $\mu$ m thick sections. Thin sections were stained with haematoxylin-eosin and analysed histologically to evaluate the degree of colon inflammation caused by the FITC and oxazolone solution. Other thin sections, after washing with phosphate-buffered saline (PBS), were incubated with rat anti-I-A/I-E (1:100) monoclonal antibody (BD Biosciences, San Jose, CA) or goat anti-mouse IgA (1:100) polyclonal antibody (ZYMED, San Francisco, CA) for overnight at 4 °C. The specimens were then washed in PBS, were incubated at room temperature for 2 h with FITC-conjugated anti-rat immunoglobulin made from rabbit or FITC-conjugated anti-goat immunoglobulin made from rabbit (1:30; Dako Cytomation, Denmark). The expression of dendritic cells or IgA was evaluated immunohistochemically under fluorescence microscopy.

### Statistical analysis

All data were expressed as the mean  $\pm$  SD. The results obtained from the three animal groups were analysed by either Student's *t*-test or an ANOVA using computer software. Differences were considered to be significant when  $P < 0.05$ .

## RESULTS

### Effect of FPP administration on systemic suppression of contact hypersensitivity by FITC and oxazolone

Contact hypersensitivity induced by FITC or oxazolone was suppressed by 50% and 37%, respectively, when FPP was administered (Fig. 2). In the dextrose administration group, the difference was not recognised in comparison to the non-treatment (water administration) group.

### FPP reduced IFN- $\gamma$ , IL-10 and TNF- $\alpha$ production induced by FITC or oxazolone

The plasma levels of IFN- $\gamma$ , IL-10 and TNF- $\alpha$  measured by ELISA were observed to increase by either FITC or oxazolone sensitisation (Fig. 3A–C). The FPP administration after FITC or oxazolone sensitisation decreased the IFN- $\gamma$  levels by 80% or 50% in comparison to the levels in the mice not treated with FPP. The FPP administration after FITC or oxazolone sensitisation decreased the IL-10 level by 50% in comparison to the levels in the mice not treated with FPP. The FPP administration after FITC or oxazolone sensitisation decreased the TNF- $\alpha$  level by 65% or 55% compared to the levels in the mice not treated with FPP. The FPP administration suppressed

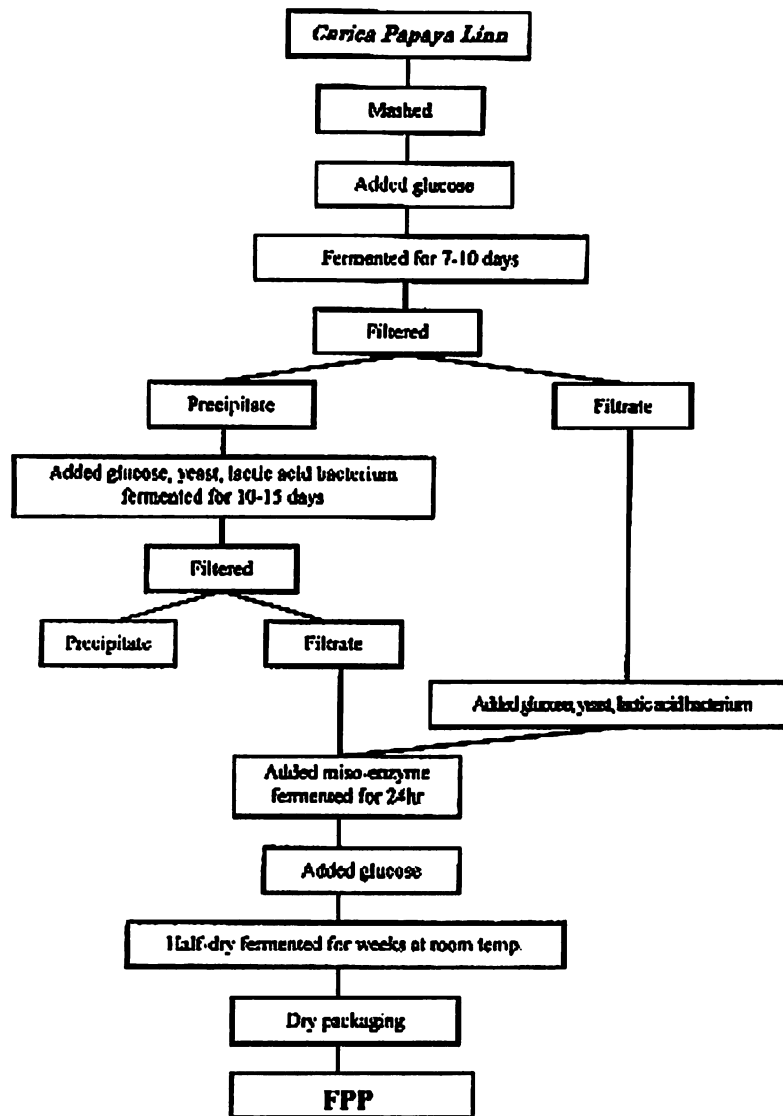


Figure 1. Manufacture of fermented papaya preparation (FPP).

cytokine increase in response to FITC or oxazolone significantly more than in the dextrose administration group.

#### Morphological and histopathological changes in FPP treated mice

Biopsy specimens were taken from the regions of positive colon reactions. The morphological observations are shown in Fig. 4. Remarkable oedema and congestion were observed due to contact hypersensitivity by FITC or oxazolone. Microscopic observation of colon samples stained with haematoxylin and eosin revealed infiltration of large numbers of lymphocytes and neutrophils due to contact hypersensitivity by FITC or oxazolone (Fig. 5). The infiltration of smaller numbers of lymphocytes and neutrophils was observed in the colons from the FPP treated mice.

#### Immunohistological changes in the FPP-treated mice

The expression of IgA and dendritic cells was observed in the colon from FPP treated animals. The colonic

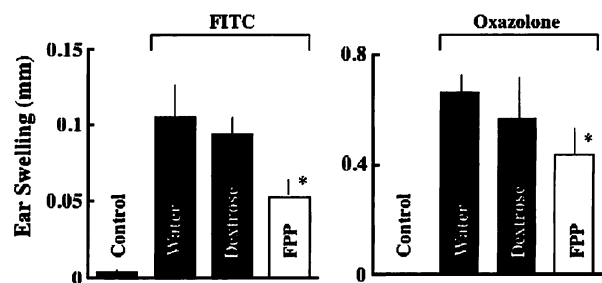
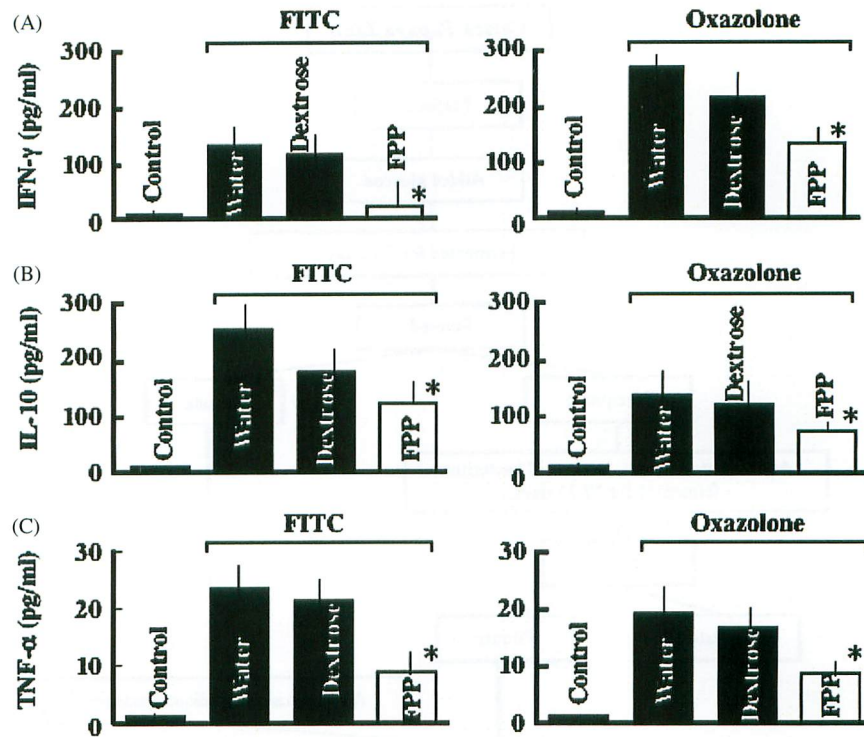
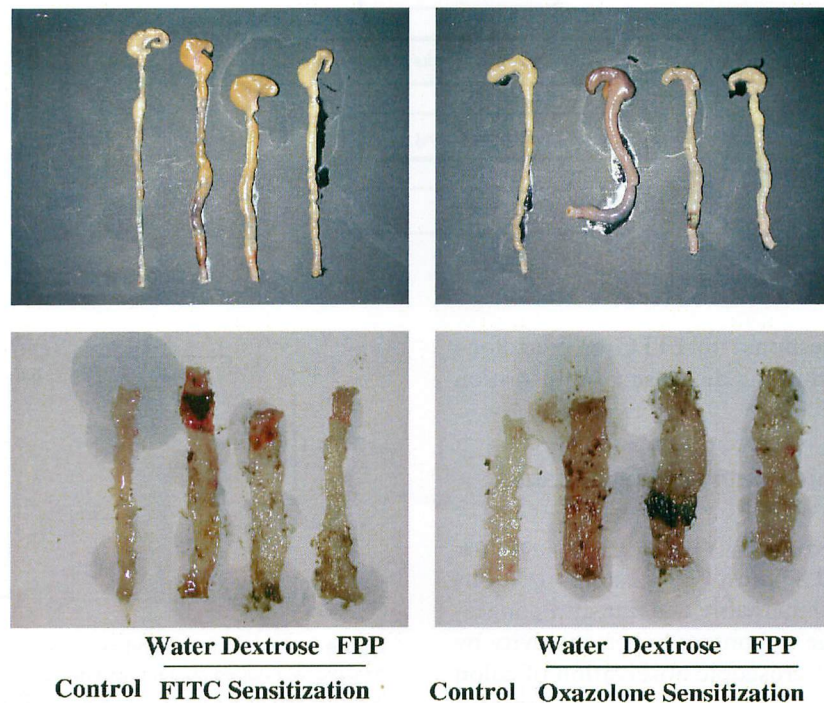


Figure 2. Effects of FPP and dextrose administration on FITC- or oxazolone-induced contact hypersensitivity. Contact hypersensitivity is expressed as the amount of ear swelling 24 h after FITC or oxazolone challenge. The data are presented as the mean  $\pm$  SD from six animals. \* $P < 0.05$  in comparison to the controls given water only.

expression of IgA markedly increased after either the FITC or oxazolone challenges. As shown in Figs 6 and 7, the expression of IgA and dendritic cells in the contact hypersensitivity groups decreased in the FPP treated mice. In addition, there was little expression in the FPP treated animals in comparison to the dextrose-treated animals.



**Figure 3.** Effects of FPP and dextrose administration on the plasma concentration of IFN- $\gamma$  (A), IL-10 (B), or TNF- $\alpha$  (C). Twenty-four hours after challenge with FITC or oxazolone, the plasma concentration of each cytokine was determined. The data are presented as the means  $\pm$  SD from six animals. \* $P < 0.05$  in comparison to the controls given water only.



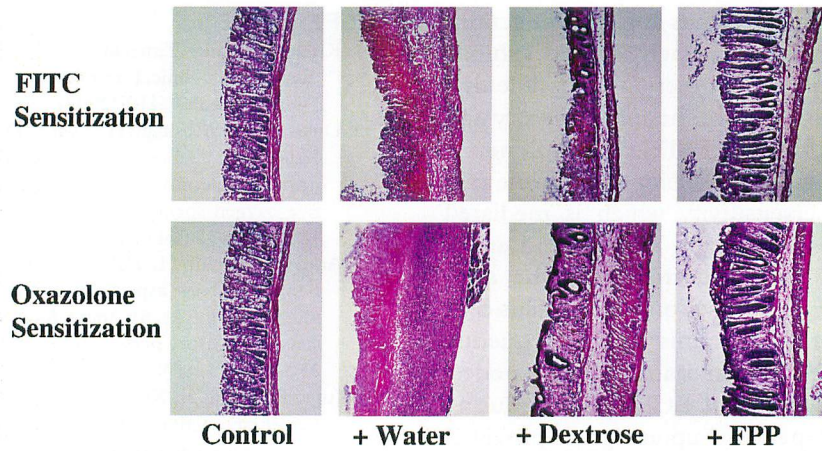
**Figure 4.** Effects of FPP and dextrose administration on FITC- or oxazolone-induced contact hypersensitivity. Contact hypersensitivity is expressed as colon oedema 24 h after FITC or oxazolone challenge.

**DISCUSSION**

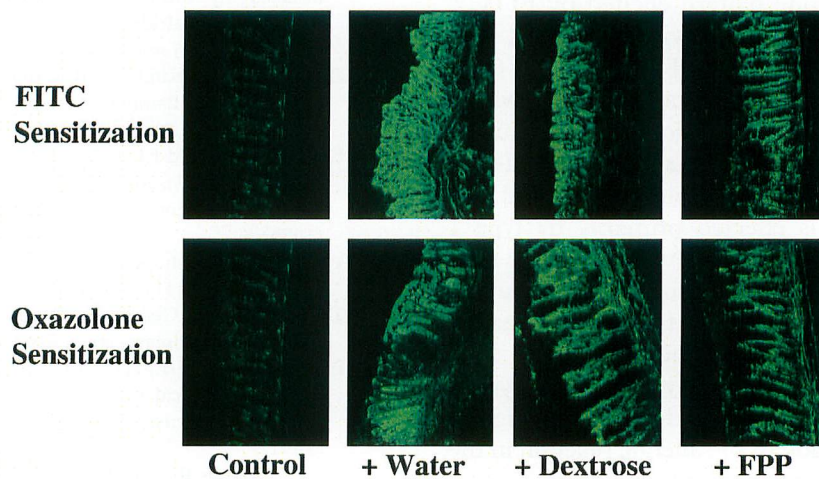
Delayed-type allergic reactions, such as type IV allergy, are often accompanied by serious inflammation. In the present study, both FITC and oxazolone successfully increased ear and colon thickness at 24 h after challenge. Furthermore, histopathological observation

of the ear and the colon sections revealed that oedema and infiltration of inflammatory cells occurred simultaneously in those mice.

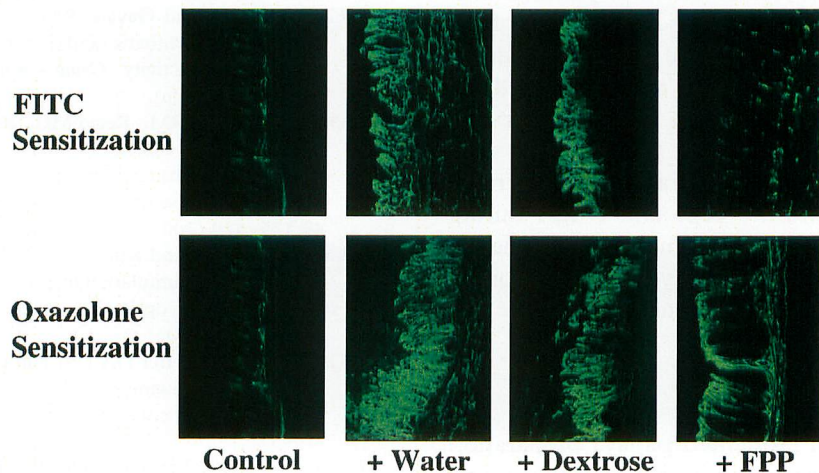
A previous study reported that the overproduction of TNF- $\alpha$  is suppressed by oral administration a FPP. TNF- $\alpha$  is a pleiotropic cytokine, originally identified by its anti-tumour activity,<sup>7</sup> and is believed to play



**Figure 5.** Effects of FPP and dextrose administration on FITC- or oxazolone-induced contact hypersensitivity. The mice were killed 24 h after FITC or oxazolone challenge and colon specimens were frozen, cut into thin sections, and then were stained with HE. Magnification,  $\times 100$ .



**Figure 6.** Effects of FPP and dextrose administration on FITC- or oxazolone-induced contact hypersensitivity. Twenty-four hours after the FITC or oxazolone challenge, the mice were killed and colon specimens were frozen, cut into thin sections, treated with anti-IgA antibody and then stained with FITC-conjugated second antibody. Magnification,  $\times 200$ .



**Figure 7.** Effects of FPP and dextrose administration on FITC- or oxazolone-induced contact hypersensitivity. Twenty-four hours after the FITC or oxazolone challenge, the mice were killed and colon specimens were frozen, cut into thin sections, treated with anti-I-A/I-E antibody and then stained with FITC-conjugated second antibody. Magnification,  $\times 200$ .

a role in many immunological and inflammatory reactions.<sup>8,9</sup> In addition, blocking TNF- $\alpha$  has also been reported to inhibit allergic skin dermatitis. For example, treatment with an anti- TNF- $\alpha$  antibody can

inhibit contact hypersensitivity,<sup>10</sup> arthus reaction<sup>11</sup> and type I allergies.<sup>12</sup> Mediators such as TNF- $\alpha$ , induced by ROS, initiate and amplify inflammatory responses.<sup>13,14</sup> FPP contains antioxidants such as

vitamin C or  $\beta$ -carotene. FPP has antioxidant actions against free radical scavenging activity and inhibits lipid peroxide formation.<sup>4</sup> It is therefore possible that drinking FPP can thus improve the inflammatory and allergic conditions induced by FITC or oxazolone.

The topical application of oxazolone to murine ears induced contact hypersensitivity which is mediated by leukotrienes, prostaglandins, TNF- $\alpha$  and other related cytokines and may contribute to the anti-allergic activity. During sensitisation, Langerhans cells (LCs) migrate from the epidermis and subsequently accumulate as dendritic cells in draining lymph nodes. In addition, the stimulation of LC migration during skin sensitisation is dependent upon signals provided by the epidermal TNF- $\alpha$ .<sup>15</sup> The oral administration of FPP may cause impaired LC migration thus resulting from the inhibition of TNF- $\alpha$  production.

However, the cytokine pattern elicited by FITC<sup>16</sup> differs from that observed following treatment with oxazolone, where a preferential IL-4/IL-10 (type 2) profile is observed.<sup>5</sup> The data contained within this report reveal that not only does FITC induce a Th2-type immunoresponse, but also a delayed-type hypersensitivity reaction. It is generally accepted that contact sensitisation is effected primarily by type 1 cells and associated cytokines.<sup>17,18</sup> It is clear that type 2 cells and their cytokine products may play an important role in the elicitation of contact dermatitis reactions.<sup>5</sup> Therefore, it was thought that the FPP could control allergic reactions by decreasing type 2 cytokine secretion. However, the details of the signals involved in the inhibition of the allergic reaction in the FPP treated animals remains to be elucidated.

In the delayed-type gastrointestinal allergy, both Th1- and Th2-type cytokines were up-regulated during contact hypersensitivity of skin.<sup>19,20</sup> IgA which is a representative of the intestinal immunity is included in the Th2 reaction, and secretory IgA (S-IgA) of the intestine increases.<sup>21</sup> The epithelial M cells residing in follicle-associated epithelium and intestinal dendritic cells underneath the epithelium are necessary to produce this IgA.<sup>22,23</sup>

It was thought that FPP restrained the increase in the number of dendritic cells and S-IgA during hypersensitivity, while also reducing the allergic reaction. However, further study is called for to elucidate the detailed mechanism of action.

## CONCLUSION

The administration of fermented papaya preparation (FPP) inhibited two types of contact hypersensitivity models, FITC (Th2 type) and oxazolone (Th1 type). Biochemical analyses revealed that the FPP decreased the elevation of inflammatory cytokine levels in plasma and of ear swelling, and of IgA, dendritic cells and inflammatory cells expression in the colon. These results suggest that FPP may therefore have a therapeutic potential for the prevention of contact hypersensitivity.

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