Author's response to reviews

Title: Saccharomyces cerevisiae legume fermented product modulates cutaneous atopic dermatitis-like inflammation in mice

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Author's response to reviews: see over
Dear Prof. Rowles:

Thank you very much for your comments for our manuscript 1989537614108090 entitled “Saccharomyces cerevisiae legume fermented product modulates cutaneous atopic dermatitis-like inflammation in mice”. We followed your instruction and in this resubmitted version, we provided our previous results of the immunomodulation effects of SCLFP in mouse splenocytes (as supplementary Figure 1, see below). The splenocytes were isolated from the mice fed with indicated dose of SCLFP five days a week for four weeks. The splenocytes were stimulated with 1 µg concanavalin A for three days, the culture supernatant were collected for cytokine determination. Interferon-gamma and IL-10 secretion were significantly increased dose-dependently in splenocytes from SCLFP fed mice compared to control (water fed) mice. But, IL-2 and IL-4 production in stimulated splenocytes were unaffected by SCLFP feeding. These results indicated that the SCLFP we tested had immuno-modulating effect in mice model. Therefore, we selected the skin sensitization model to test the anti-inflammatory effects of SCLFP.

Figure S1. Immunomodulatory effect of SCLFP feeding in mouse splenocyte. Mice were fed with indicated dose of SCLFP five days per week. After four weeks, the mice were sacrificed and the single cell suspensions of spleen were prepared for concanavalin A stimulation for three days. The cultured supernatant was collected for IFN-γ (A), IL-10 (B), IL-2 (C), and IL-4 (D) ELISA. The data were represented as mean of concentration ± standard deviation. Significant differences compared between different group using t-tests were indicated by asterisks (*p<0.05 and ***p<0.001). This supplementary figure legend was added in page 25 to 26.
Response to editor:

**In abstract:**
The following sentences were added in page 2, line 26 to 30 (underlined, red color) to explain the correlation of SCLFP used in this study. “In a preliminary study, we found that feeding with *Saccharomyces cerevisiae* legume fermented product (SCLFP) could modulate mouse splenocytes to produce IFN-γ and IL-10. Therefore, we selected the cutaneous atopic dermatitis-like inflammation induced by epicutaneous sensitization in mice to test the anti-inflammatory effects of SCLFP in this study.”

**In Methods:**
The methods used in immunomodulatory effects of SCLFP were added in page 7 line 107, page 8 line 112 to 115 and page 10 line 160 to 161 (underlined, red color).

**In Results:**
The following sentence was added in page 11, line 167 to 175 (underlined, red color). Immunomodulatory effects of SCLFP administration

The splenocytes were isolated from the mice fed with indicated dose of SCLFP (3, 6 or 15 mg) five days a week for four weeks. The splenocytes were stimulated with 1 µg concanavalin A for three days, the culture supernatant were collected for cytokine determination. Interferon-gamma and IL-10 secretion were significantly increased dose-dependently in splenocytes from SCLFP fed mice compared to control (water
fed) mice. But, IL-2 and IL-4 production in stimulated splenocytes were unaffected by SCLFP feeding. These results suggested that SCLFP feeding could exert immune-modulatory effects that drive immune response toward Th1 and immunosuppressive response. Therefore, the Th2 epicutaneous sensitization mice model was selected to test this hypothesis.

Antigen-specific response results were added in page 13 line 198 to 200.

Sincerely,

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