Epicutaneous sensitization with protein allergens differentiates naïve T cells into not only Th2 but also Th17 cells, which may be involved in delayed reactions in protein contact dermatitis

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Introduction

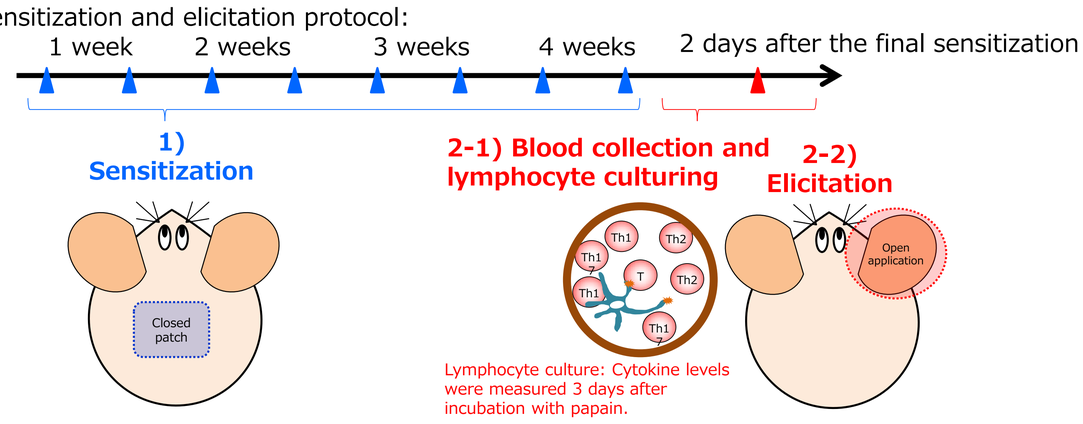
It is well known that sensitization with chemical allergens differentiates naïve T cells into Th1 cells in the draining lymph nodes, thereby eliciting delayed-type hypersensitivity reactions. IFNγ secreted by Th1 cells plays an important role in this process. However, the types of effector T cells that are differentiated in the draining lymph nodes of mice sensitized with protein allergens remain unknown. Furthermore, the types of hypersensitivity reactions elicited by protein allergens remain unclear. To address these issues, we investigated mice sensitized with papain enzyme as a protein allergen.

Study purpose:

To clarify the types of skin allergy induced by papain sensitization

Methods

Sensitization and elicitation protocol:



1) Sensitization: Protease papain (Calbiochem) was applied to the back skin of hairless mice (HOS:HR-1) in a closed patch (100 µL of 0.1% papain). The application was performed twice weekly, for 4–5 weeks (total number of applications = 8–10).

2-1) Blood collection and lymphocyte culturing: Two days after the final sensitization, the blood samples were collected and lymph nodes (axillary lymph node and inguinal lymph node) were harvested. The concentration of papain-specific antibodies in the blood samples was measured using enzyme-linked immunosorbent assay (ELISA). Lymphocytes were cultured with papain, following which, the levels of Th1 cytokine (IFNγ), Th2 cytokine (IL-4), and Th17 cytokine (IL-17) were measured using ELISA and real-time PCR. In addition, the expression of transcription factors Th1 (T-bet), Th2 (GATA-3), and Th17 (RORγt) was measured using real-time PCR. In addition, Th1/Th2/Th17 phenotyping was carried out using Th1/Th2/Th17 phenotyping Kit (BD bioscience) by flow cytometer.

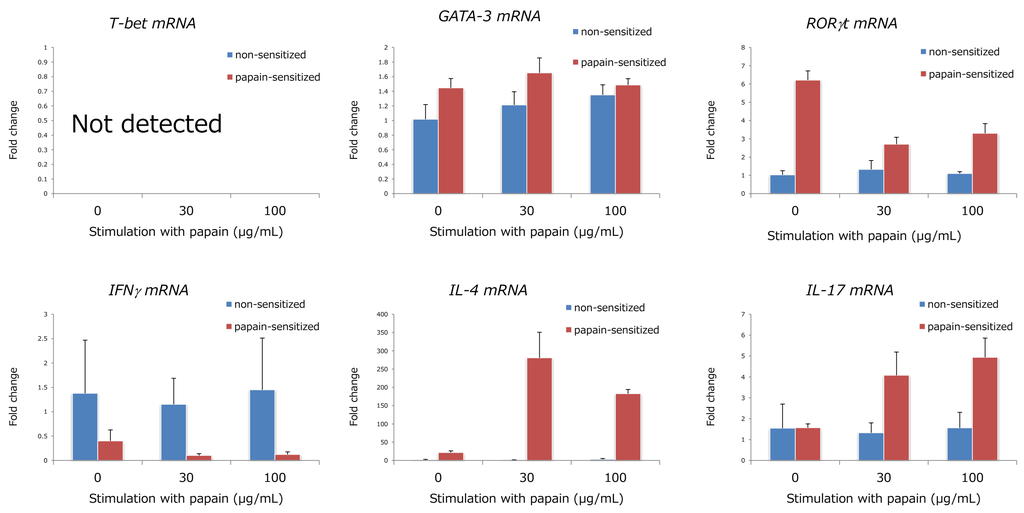
2-2) Elicitation: To this end, 25 µL of 1% papain was topically applied to the right ears of papain-sensitized and non-sensitized mice. The vehicle (0.2% Tween-20) was applied to the left ears. Before elicitation, and 3, 24, 48, and 96 h after elicitation, the following experiments were performed:

- Measurement of ear swelling using a micrometer
- Hematoxylin–eosin staining of ear skin samples
- Measurements of Th1, Th2, and Th17 cytokine levels using real-time PCR
- Immunostaining of RORγt using a specific antibody
- Giemsa staining of ear skin samples

Results

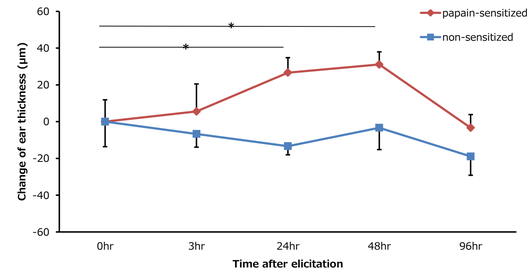
[Exp.1] Effector T cells in the draining lymph nodes

The levels of the Th2 transcription factor, GATA-3, and the Th17 transcription factor, RORγt, were up-regulated in the lymphocytes of papain-sensitized mice. Antigen stimulation induced *de novo* synthesis of IL-4 and IL-17 in the lymphocytes of papain-sensitized mice. These results demonstrate that naïve T cells differentiate into Th2 and Th17 following papain sensitization.



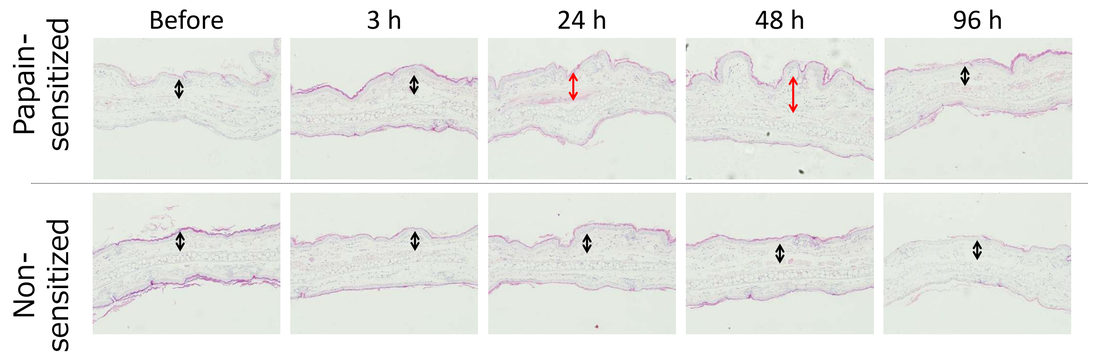
[Exp.2] Ear swelling following elicitation

Following elicitation, the skin of the ear thickened in the papain-sensitized mice but not in the non-sensitized mice.



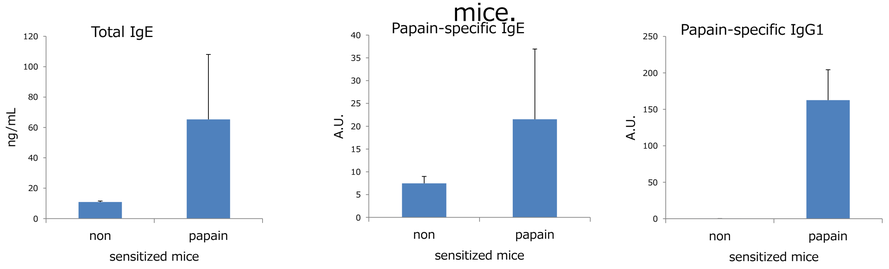
[Exp.3] Hematoxylin–eosin staining of the ear skin

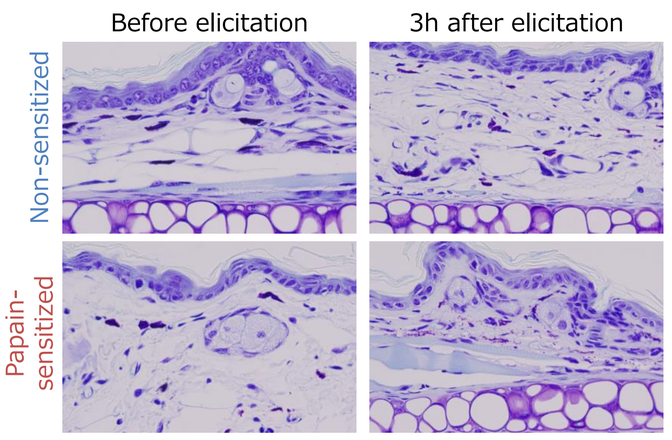
The side of the ear (backside) wherein papain was applied showed swelling in papain-sensitized mice following elicitation (the red arrow in comparison with the black arrow).



[Exp.4] Antigen-specific antibodies

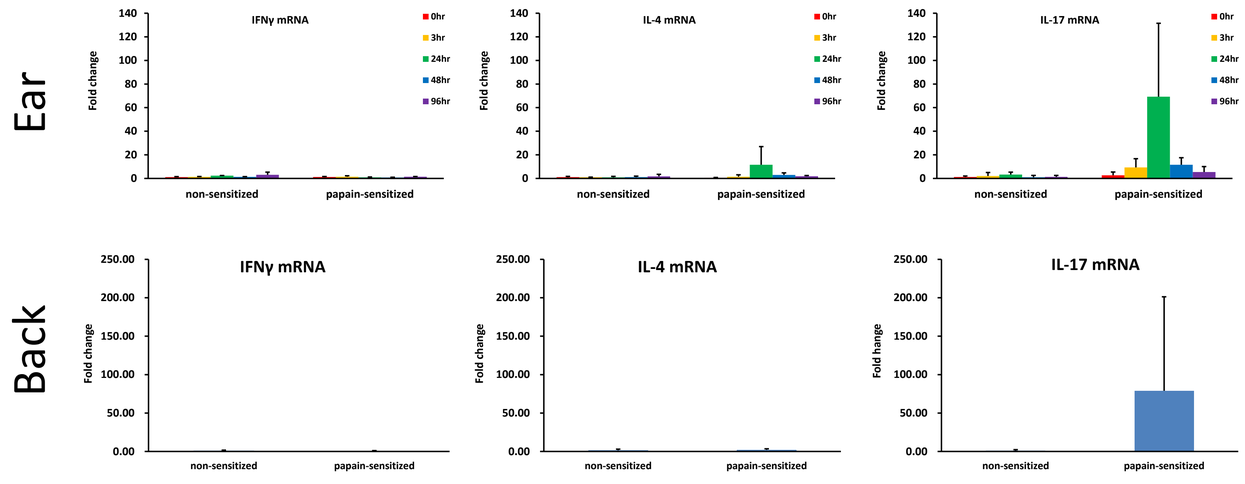
The concentration of papain-specific antibodies increased in papain-sensitized mice.





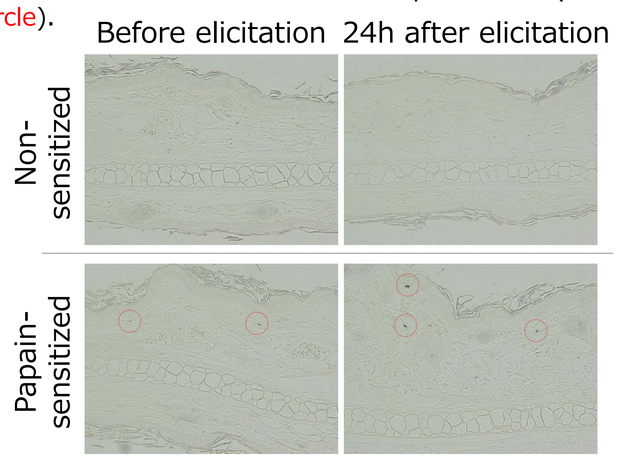
[Exp.5] Th1, Th2, and Th17 cytokine levels in the ear and back

IL-17 mRNA was upregulated in papain-sensitized mice 24–48 h after elicitation. These results indicated that antigen-specific Th17 cells infiltrated and activated the skin.



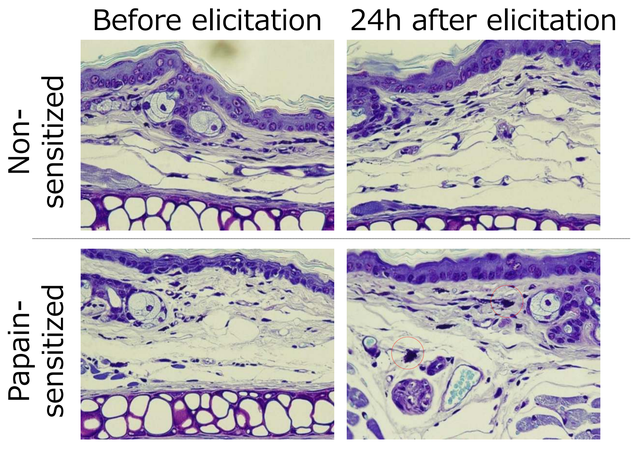
[Exp.6] Th17 transcription factor in the ear

RORγt-positive cells were observed in papain-sensitized mice, and further increased after elicitation. These results indicate that Th17 cells infiltrated in the skin of papain-sensitized mice and were activated by elicitation (red circle).



[Exp.7] Giemsa staining of the ear

The number of cells with Giemsa-positive granules in the cytosol increased following elicitation (red circle).



Conclusion

Papain sensitization causes naïve T cells to differentiate into Th17 as well as Th2 cells as effector T cells that contribute to allergic reactions.

The generated Th2 cells produce IL-4 and are involved in IgE synthesis. Conversely, the generated Th17 cells migrate into the skin and produce IL-17 following elicitation.

Further investigations are required to clarify the skin allergy caused by Th17 cell (contact dermatitis?) and to develop the associated safety assessments (Buehler test?).