

Chondrex, Inc. introduces a new protocol to induce nasal hypersensitivity in mice. Dr. Saika *et al.*, published this model using Chondrex Inc's anti-OVA IgE monoclonal antibody, E-C1, in C57BL/6 mice (1). Because many transgenic mouse strains are available, this model can be adapted to investigate the role of gene function in the development of allergic reactions. The evaluation methods are simple and do not require sacrificing mice. As a result, multiple evaluations can be conducted as well as an endpoint study, allowing continual evaluation of the entire study period. The following information is a summary of the protocol and results. Please contact Chondrex, Inc if you are interested in the anti-OVA IgE monoclonal antibody (Catalog # 3006).

Materials and Methods

Experimental Animals

C57BL/6Jcl male mice were used after one week of preliminary breeding experiments in SPF conditions at room temperature $23 \pm 3^\circ\text{C}$, humidity 30 to 90%, lighting time 7:00 to 21:00, and allowed to ingest solid food and water ad libitum.

Whole-body Sensitization Model

The mouse allergic rhinitis model was induced using egg white albumin (OVA) as the allergen. The mice received 2 mg of Aluminum adjuvant containing 75 μg of OVA in PBS intraperitoneally on days 0, 7, and 14. Then the mice received 20 μl of PBS containing 250 μg of OVA in both nasal passages every day from day 21 to 27 (OVA/OVA group). 24 hours after the last sensitization, serum and tissue samples were collected from mice (Figure 1A). Negative control mice received only PBS administered intraperitoneally/intranasally (PBS/PBS group).

Nasal Hypersensitivity Model

Mouse anti-OVA-specific IgE antibodies (Clones E-C1 and E-G5, Catalog # 3006 and 3007, respectively, Chondrex, Inc.) were used. Mice received E-C1 (2 μg or 10 μg) or E-G5 (2 μg) in PBS, intravenously, on days 0 and 1. Then the mice received 20 μl of PBS containing 250 μg of OVA in both nasal passages every day from day 1 to 7 (OVA group) (Figure 1B). Negative control mice received only PBS administered intravenously/intranasally (PBS group).

Evaluating Symptoms

After the final intranasal sensitization, the number of times that nasal scratching and sneezing occurred was measured for 10 minutes.

Measuring Serum OVA-specific IgE

Sera was collected at day 28 in the whole-body sensitization models and day 8 in the nasal hypersensitivity models. Mouse serum anti-OVA IgE levels were analyzed with an ELISA kit.

Histological Evaluation

The heads were fixed in 4% paraformaldehyde, demineralized with 0.5 M ethylenediamine tetraacetic acid for 1 week. The samples were embedded in paraffin and sliced 6 μm thick to make slide samples.

The slides were stained with Periodic acid Schiff (PAS), and the number of goblet cells in a 100 μm wide section of basement membrane were counted in three arbitrarily selected regions of the nasal septum mucosa. In addition, the thickness of the nasal mucosa was measured in three randomly selected regions.

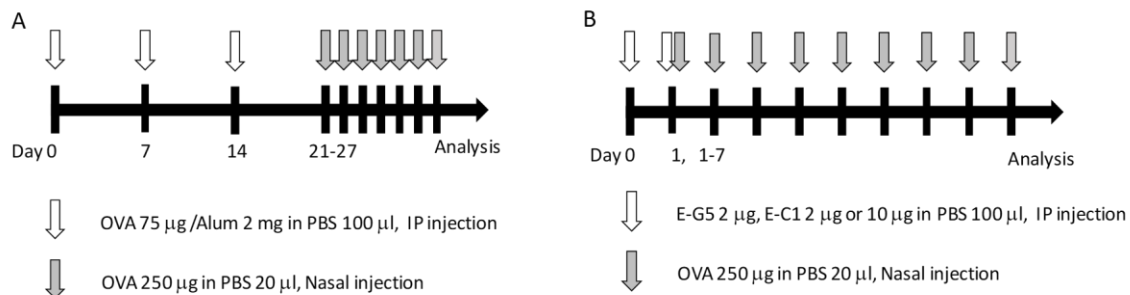


Figure 1. Immunization protocols

A: Whole body sensitization model and **B:** Nasal hypersensitivity model

Results

In the whole-body sensitization model, the number of sneezing incidents was significantly higher in the OVA/OVA group (21.0 ± 7.1 times) compared to the PBS/PBS group (4.8 ± 1.5 times) (Figure 2A). The number of nasal scratching incidents tended to be higher in the OVA/OVA group (37.8 ± 12.2 times) than in the PBS/PBS group (28.8 ± 37.8 times) (Figure 2C). In addition, the mice who received OVA sensitization and PBS nasal administration showed no nasal allergy symptoms (data not shown).

In the nasal hypersensitivity model, the number of scratching and sneezing incidents in the mice who received 10 μg E-C1 were higher than the three other groups who received 2 μg E-C1, 2 μg E-G5, and PBS (Figure 2B and 2D).

In the histological analysis, the OVA/OVA group in the whole-body sensitization model showed an increased number of goblet cells in the 100 μm wide section of basement membrane (12.7 ± 1.0) compared to the PBS/PBS group (7.4 ± 1.4) (Figure 3A and Figure 4E). The OVA/OVA group also had thickened nasal mucosa ($25.7 \pm 6.1 \mu\text{m}$) compared to the PBS/PBS group ($16.3 \pm 0.6 \mu\text{m}$) (Figures 3A, 3C, 4E, and 4G). However, in the model of nasal hypersensitivity, neither increased goblet cell number nor thickening of the nasal mucosa was observed even in the OVA-IgE intravenous group (Figures 3B, 3D, 4F, and 4H).

Furthermore, serum OVA-specific IgE antibodies were detected in the OVA/OVA group ($11.3 \pm 9.6 \text{ ng/ml}$), but not in the PBS/PBS group. Interestingly, serum OVA-specific IgE was not detected

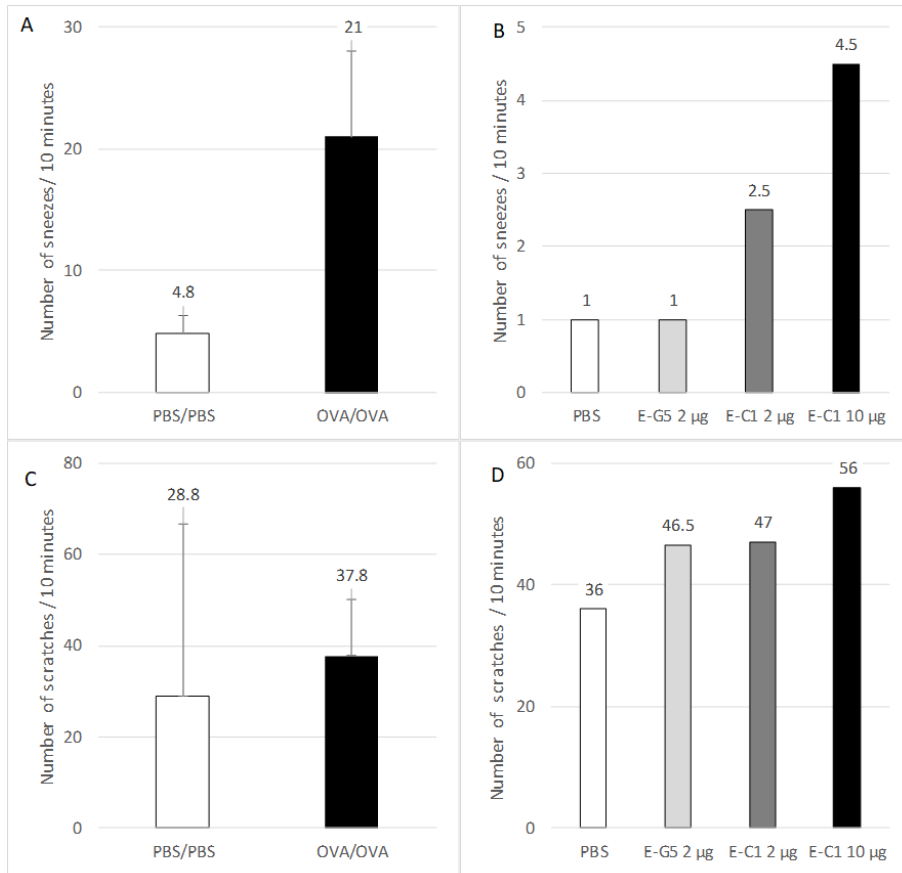


Figure 2. Nasal symptoms in the whole-body sensitization model and the IgE passive sensitization model.

Nasal symptoms in the whole body sensitized model after intranasal OVA sensitization: number of sneezes (A), number of nasal scratches (C). Nasal symptoms in the IgE passive sensitization model after intranasal OVA sensitization: number of sneezes (B) and number of nasal scratches (D). The whole body sensitization model was tested in the PBS/PBS group (n = 4) and the OVA/OVA group (n = 8). The IgE passive sensitization model was tested in the PBS group (n = 2), E-G5 2 μg group (n = 2), E-C1 2 μg group (n = 2), and E-C1 10 μg group (n = 2)

(* P < 0.05, Mann-Whitney U Test)

in the nasal hypersensitivity model (data not shown).

Summary

In the whole-body sensitization model, OVA sensitization showed an increased number of nasal scratching/sneezing incidents, an increased serum OVA-specific IgE antibody titer, and an increased number of goblet cells. These results indicate that in the immediate phase of this allergic rhinitis model, local changes in the nasal mucosa are induced by establishing an acquired immune response (IgE antibody production) against OVA.

On the other hand, the new IgE passive sensitization model (nasal hypersensitivity model) is induced by injecting antigen-specific IgE monoclonal antibodies with subsequent sensitization of the local area. In nasal hyperresponsiveness patients, the antigen usually cannot be identified as an allergen in the early stages of sensitization. Therefore, an antigen-specific IgE antibody, which can be captured by the local mast cells, was used to mimic this disease stage. When the antigen specific IgE

antibodies were intravenously injected, the IgE antibodies were captured by the local mast cells, activating the mast cells when antigens from the nasal cavity bound to the captured IgE antibodies on the mast cell.

In both models, the immediate phase could be induced by intranasal sensitization of the antigen. Furthermore, in the nasal hypersensitivity model, serum OVA IgE antibodies, injected on days 0 and 1, were not detected in the serum samples on day 7, because the half-life of IgE antibodies in the blood is 12 hours. In addition, no histological changes occurred in this model. This suggests that the immediate phase may be induced depending on IgE bound mast cells and basophils on the mucosal surface as localized mast cells capture IgE antibodies from the bloodstream.

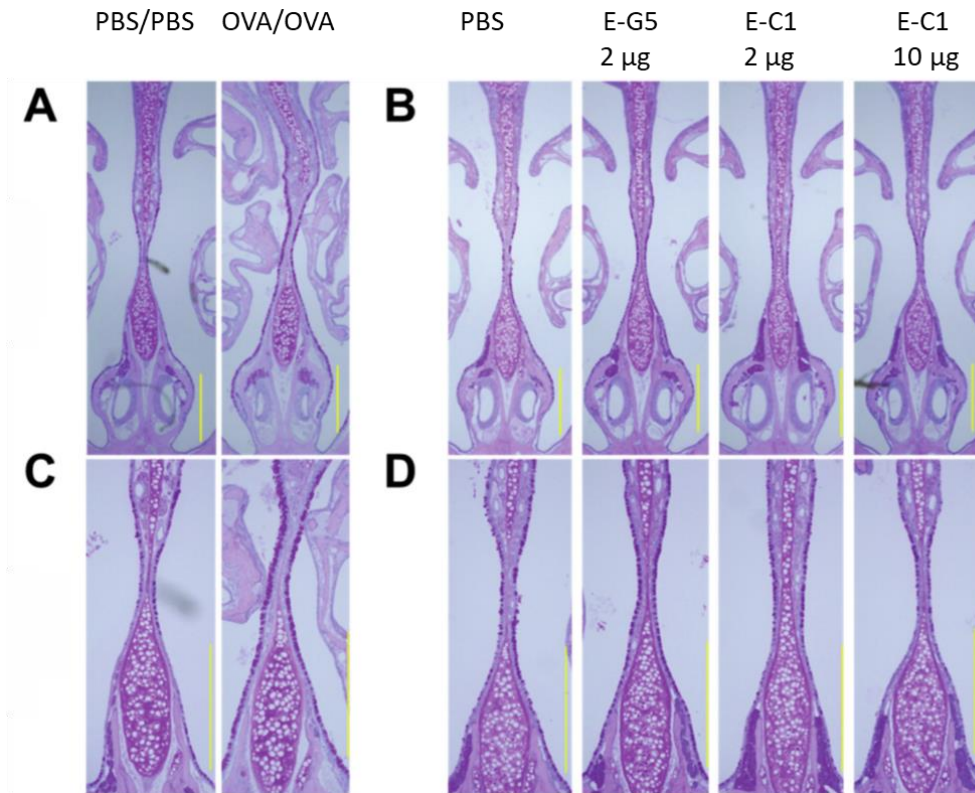


Figure 3. Histological evaluation with PAS staining of the whole body sensitization model and the IgE passive sensitization model.

Coronary fracture of the nasal mucosa in the whole body sensitization model (A) and (C) and in the IgE passive sensitization model (B) and (D).

As described above, this nasal hypersensitivity model reflecting the immediate phase of allergic rhinitis was developed using the correlation of 1) invading allergens from mucosal membranes, 2) allergen-specific IgE antibodies, and 3) mast cells. In recent years, patients with early stage allergic rhinitis without elevated allergen-specific serum IgE antibody levels have been considered Local Allergic Rhinitis (LAR) patients. We believe that this model can analyze the pathological condition of LAR and evaluate the role of the immediate phase in allergic rhinitis. In addition, the whole-body sensitization model takes 1 month to analyze,

although the nasal hypersensitivity model can proceed with analysis after only 8 days.

This model is convenient and useful for analyzing the immediate phase via IgE-dependent antigen-antibody reactions in early stage allergic rhinitis.

Reference

1) New Protocol for a mouse nasal hypersensitivity model, *Kawasaki Medical Journal* 43(2):109-117 (2017) doi:10.11482/KMJ-J43(2)109

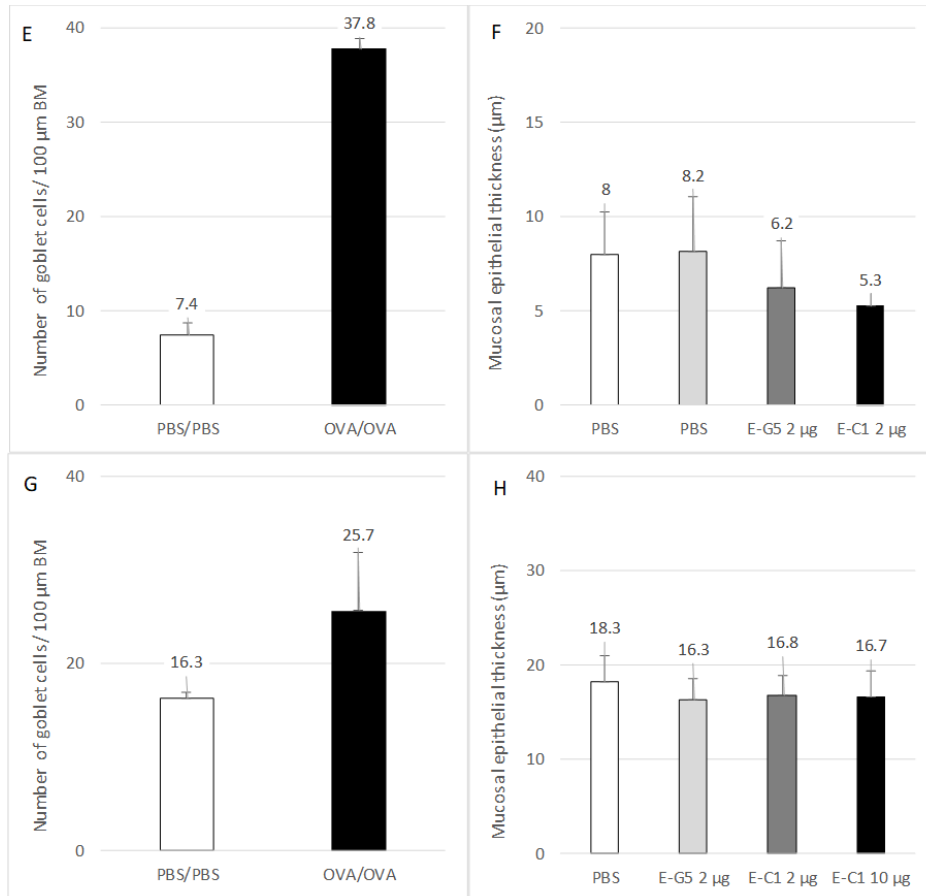


Figure 4. Histological evaluation of the whole-body sensitization model and the IgE passive sensitization model.

The number of goblet cells in a 100 μ m wide section of the basement membrane of the nasal septum mucosa (E), and epithelial cell thickness (G) in the whole body sensitization model. The number of goblet cells in a 100 μ m wide section of the basement membrane of the nasal septum mucosa (F), and epithelial cell thickness (H) in the IgE passive sensitization model. The number of nasal mucosal goblet cells and thickness of nasal mucosal epithelium were shown as the average of 3 randomly selected sites. (*P < 0.05, Mann-Whitney U Test)