A Mouse Nasal Hypersensitivity Model

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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 complete 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 (Cat # 3006).

MATERIALS AND METHODS

Experimental Animals

C57BL/6JJcl male mice were acclimated for a week in Specific Pathogen Free conditions at room temperature (23 ± 3°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, Cat # 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/OVA group) (Figure 1B). Negative control mice received only PBS administered intravenously/intranasally (PBS/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

Serum was collected on 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 then 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.

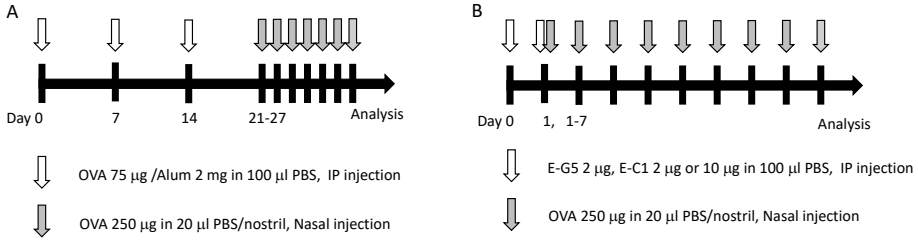

Figure 1. Immunization protocols
A: Whole body sensitization model and B: Nasal hypersensitivity model

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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 (OVA/PBS data not shown).

In the nasal hypersensitivity model, the number of scratching and sneezing incidents in the mice who received 10 µg E-C1 was higher than the three other groups who received 2 µg E-C1, 2 µg E-G5, or 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 cells) compared to the PBS/PBS group (7.4 ± 1.4 cells) (Figure 3A and Figure 4E). The OVA/OVA group also had thickened nasal mucosa (25.7 ± 6.1 µm) compared to the PBS/PBS group (16.3 ± 0.6 µm) (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, in the whole-body sensitization model, serum OVA-specific IgE antibodies were detected in the OVA/OVA group (11.3 ± 9.6 ng/ml), but not in the PBS/PBS group. In the nasal hypersensitivity model, no serum OVA-specific IgE antibodies were detected in either the OVA/OVA or the PBS/PBS groups which is interesting given that the half-life of injected IgE is supposed to be 12 hours (data not shown).

SUMMARY

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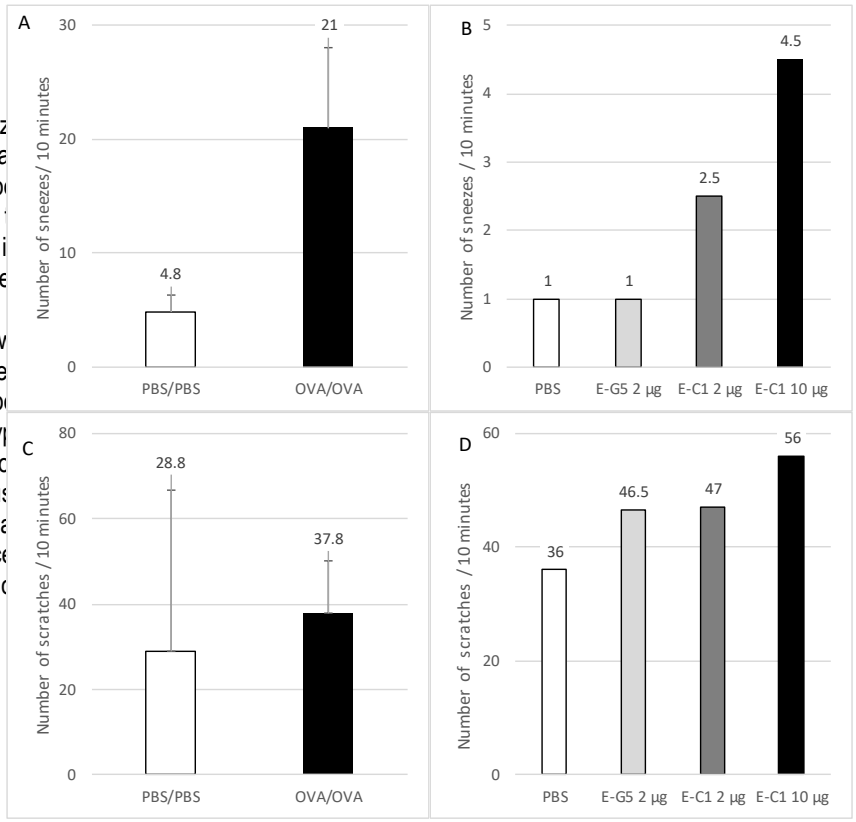

Figure 2. Nasal symptoms in the whole-body sensitization model and the IgE passive sensitization model.
Nasal symptoms in the whole body sensitized model after intranasal OVA sensitization: number of sneezes (A), number of nasal scratches (C). Nasal symptoms in the IgE passive sensitization model after 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)

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In both models, the immediate phase could be induced by intranasal sensitization of the allergens. 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 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.

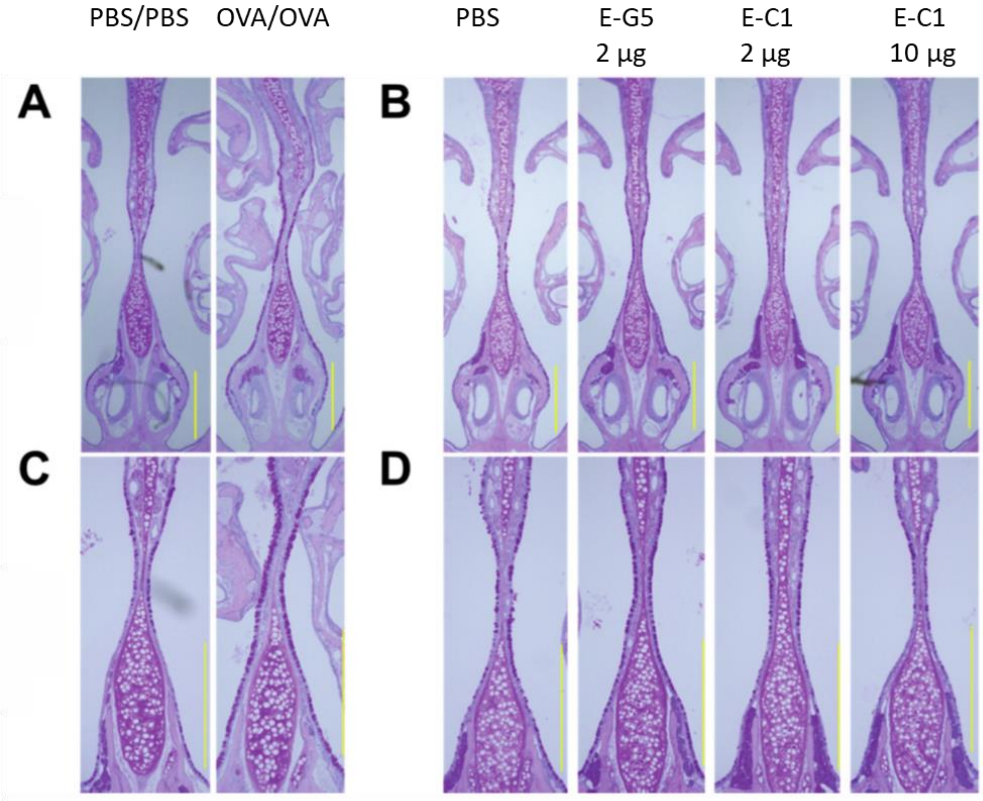

Figure 3. Histological evaluation with PAS staining of the whole body sensitization model and the IgE passive sensitization model.
Coronary fracture of the nasal mucosa in the whole body sensitization model (A) and (C) and in the IgE passive sensitization model (B) and (D).

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, early stage allergic rhinitis without elevated allergen-specific serum IgE antibody levels have been considered Local Allergic Rhinitis (LAR). Chondrex, Inc. believes 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.

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

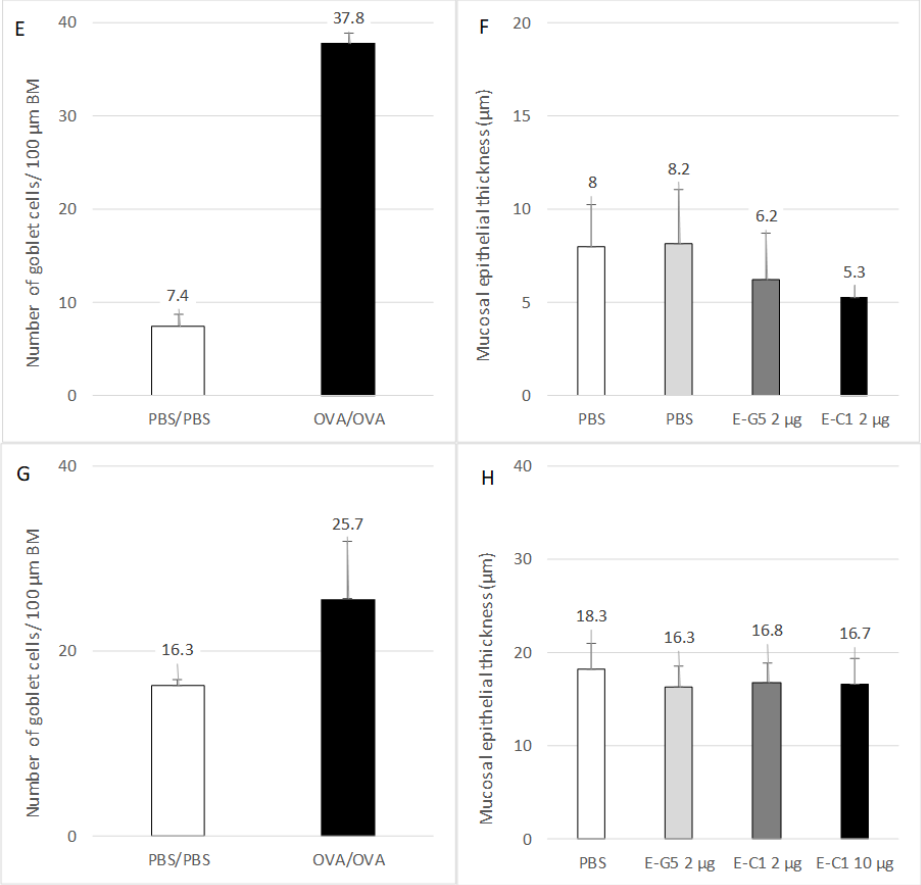

Figure 4. Histological evaluation of the whole-body sensitization model and the IgE passive sensitization model.
The number of goblet cells in a 100 μm wide section of the basement membrane of the nasal septum mucosa (E), and epithelial cell thickness (G) in the whole body sensitization model. The number of goblet cells in a 100 μm 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)

REFERENCES

1. New Protocol for a mouse nasal hypersensitivity model, *Kawasaki Medical Journal* **43**,109-117 (2017).

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