



Induction and Characterization of the Allergic Eye Disease Mouse Model

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Abstract

Ocular IgE-associated allergy ranges from mild disease (seasonal and perennial allergic conjunctivitis) to more chronic/severe and vision-threatening forms (atopic and vernal keratoconjunctivitis). Whereas mild forms of disease have been studied extensively, less is known about the more chronic forms. Our lab has helped to address this knowledge gap by developing and characterizing an allergen-induced, chronic/severe, IgE-associated model of ocular allergy referred to as the severe allergic eye disease (AED) model. It is distinct from previously described models that mimic the more mild forms, referred to in the literature as the allergic conjunctivitis (AC) model. The purpose of this method article is to detail the protocol to induce and characterize the AED model and directly compare these mice to the mild AC model. Troubleshooting and implications are also discussed.

Keywords Allergic eye disease, AED, Allergic conjunctivitis, T cells, Th2, Allergy, Conjunctivitis, Type 2 immunity, Fibrosis, Dendritic cells

1 Introduction

Ocular allergy is a set of specific allergic inflammatory conditions that affect the conjunctiva, the eyelid, and, in severe cases, the cornea. Type and severity range from mild seasonal and perennial allergic conjunctivitis (AC) to more severe and chronic such as atopic and vernal keratoconjunctivitis (AKC and VKC, respectively) [1]. While these types differ significantly in clinical symptoms, prognosis, and pathobiology, they all share a common IgE-associated etiology [1]. Hence, allergenic CD4⁺ T helper (Th)2 cells and their cytokines (IL-4, IL-5, IL-13) are central to mediate disease [2–4].

Though several species, such as the rat and guinea pig, have been used to study ocular allergy, presently the preferred species is the mouse system [1]. Several mouse models exist for studying ocular allergy that are induced by either active or passive immunization against a model antigen, such as short rag weed or ovalbumin. Until recently, most mouse models have primarily mimicked the more mild types of ocular allergy akin to AC in people. These models present with classical immediate hypersensitivity signs and symptoms that include swelling of the bulbar conjunctiva (i.e., chemosis), superficial conjunctival vasodilation (i.e., redness), tearing (Fig. 2), and lid edema [2, 5].

By contrast, AKC and VKC are more chronic and severe diseases that have more complex mechanisms and can develop tissue remodeling [6–11], although the pathobiology is less understood. To help address current knowledge gaps [3, 12], our lab established a model of chronic ocular allergy, which we refer to as the allergic eye disease (AED) model so as to differentiate it from the classical AC model. Mice with AED present with the more severe clinical manifestations that are present in patients with AKC and VKC [13–15]. At a clinical level, manifestation of conjunctival fibrosis, thick mucoid discharge, and eczema of the eyelid are all present in the AED model [5, 16–18] (Fig. 2). On a cellular level, robust eosinophil recruitment occurs in the conjunctiva, and lymphangiogenesis is observed in the cornea [16, 19, 20]. Thus, the AED model now enables the study of the chronic eye allergy setting and thereby helps address major knowledge gaps in our understanding of the pathobiology.

The focus of this methods paper is the induction and analysis of AC and AED using C57BL/6 mice and ovalbumin (OVA) antigen, as outlined in Figure 1.

2 Materials

2.1 Chronic AED Model

1. 15 mL or smaller beaker.
2. Stir bar.
3. Stir plate.
4. Ovalbumin: OVA from chicken egg white.
5. Pertussis toxin (PT): Pertussis toxin from *Bordetella pertussis*.
6. Aluminum hydroxide (Imject Alum).
7. Sterile 1× PBS.
8. 1 mL syringe.
9. 25-gauge needle.
10. C57BL/6 mice (*see* **Note 1**).

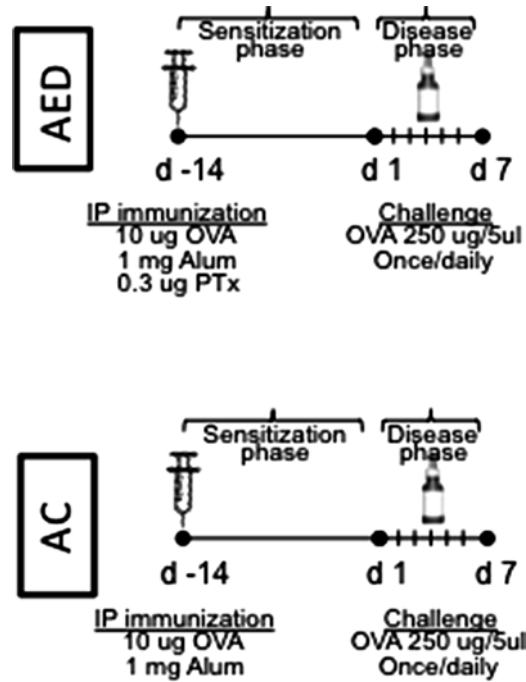


Fig. 1 Induction of mild allergic conjunctivitis (AC) and chronic allergic eye disease (AED) models. Allergy is induced in mice by IP injection of immunization mix and 14 days later topical instillation of OVA for 7 consecutive days

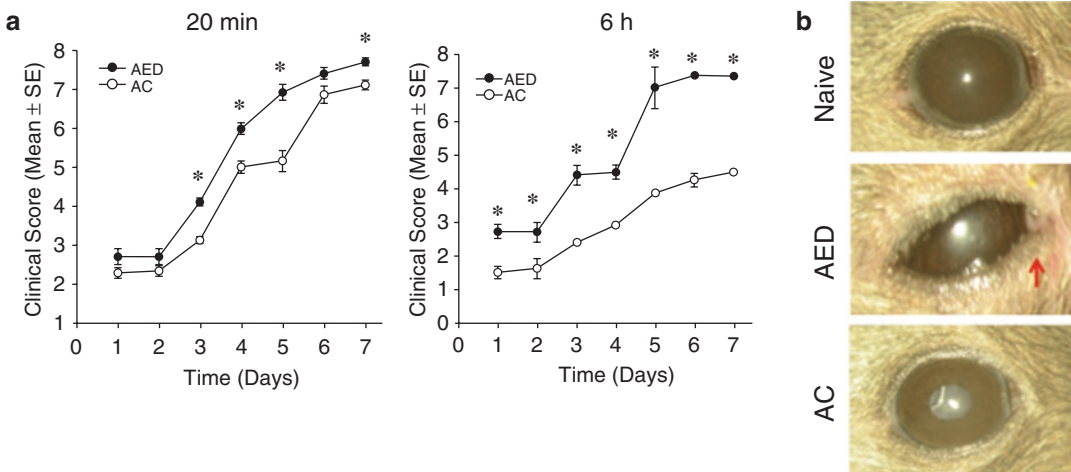


Fig. 2 AED results in more severe clinical disease compared to AC. Clinical scores were assessed at 20 min (to determine immediate hypersensitivity) and 6 h post-challenge (to determine the late-phase reaction). (a) Mice with AED have higher classic allergic clinical responses compared to mice with AC that persisted even 6 h post-challenge. (b) Severe clinical manifestations such as blepharitis (red arrow), mucoid discharge, and corneal epitheliopathy (data not shown) developed in mice with AED but were absent in mice with AC. Data is representative of five independent experiments ($n = 5$ mice/group/experiment). $p < 0.05$ was considered significant

11. Immunization mix + PT: 10 μ g of OVA, 300 ng Pertussis toxin (PT), and 1 mg (100 μ L) of Imject Alum in 100 μ L PBS per mouse (*see* **Notes 2 and 3**).

2.2 Mild AC Model

1. Ovalbumin (OVA from chicken egg white).
2. Aluminum hydroxide (Imject Alum).
3. Sterile 1 $\times$ PBS.
4. 1 mL syringe.
5. 25-gauge needle.
6. C57BL/6 mice.
7. Immunization mix: 10 μ g of OVA and 1 mg (100 μ L) of Imject Alum in 100 μ L PBS per mouse.

2.3 Topical Challenge of Mice

1. OVA Mix: 50 mg/mL OVA in 1 $\times$ PBS (vortex prior to administration).
2. Sterile 1 $\times$ PBS.

2.4 Clinical Scoring and Evaluation

1. Slit-lamp microscope (Topcon SL-D7/D8Z with Nikon Digital camera).
2. Software (Topcon IMAGE-net R4 digital imaging system).

3 Methods

3.1 Induction of AED

1. Prepare immunization + PT mix (*see* **Notes 2 and 3**). First add the 10 μ g of OVA, 300 ng pertussis toxin (PT), and 100 μ L of PBS (per mouse) into 50 mL beaker, and begin to stir the mix.
2. Once these have been added, add 100 μ L of Imject Alum slowly while continuing stirring (*see* **Note 4**).
3. Keep stirring immunization + PT mix for an hour at RT. Upon completion, the immunization mix is ready to be injected into the mice.
4. As the mixture is viscous, slowly fill the syringe with 200 μ L of immunization mix.
5. Administer a total of 200 μ L via intraperitoneal (IP) injection into the mouse using a 25-gauge needle (*see* **Note 5**).
6. Allow mouse to rest for 14 days and proceed to topical challenge.

3.2 Induction of AC

1. Make the immunization mix as described (Subheading [3.1](#)) (*see* **Note 6**).
2. Separately add 100 μ L of Imject Alum slowly to this mix while stirring.
3. Keep stirring this immunization mix for an hour.

4. Fill the syringe with 200 μL of immunization mix.
5. Administer a total of 200 μL via IP injection into the mouse with 25-gauge needle.
6. Allow mouse to rest for 14 days and proceed to topical challenge.

3.3 Topical Challenge of Mice

1. Gently hold the mouse, and apply 5 μL of the OVA mix directly onto the eye using a micropipette, without touching the surface tissues of the eye with the instrument.

3.4 Clinical Scoring and Evaluation of Allergic Reaction

1. Mice are clinically evaluated using a slit-lamp microscope at 20 min and 6 h post-topical challenge (*see* **Notes 7–9**).
2. There are four main symptoms assessed in the clinical scoring of an ocular allergic response (Fig. 2). Each is scored on a scale from 0 to 3+ (0 being absent; 3+ being severe) [2, 21] (*see* **Notes 10–12**).
 - (a) Tearing
 - (b) Lid edema
 - (c) Conjunctival vasodilation (or conjunctival redness)
 - (d) Chemosis (or bulbar conjunctival edema)
3. There are also three severe clinical manifestations that can be assessed (Fig. 2) [18] (*see* **Note 13**), which include:
 - (a) Anterior blepharitis
 - (b) Muroid discharge
 - (c) Corneal epitheliopathy (*see* **Note 14**)
4. On the last day of challenge (i.e., day 7), blood can be collected to assess IgE levels (Fig. 3), and cervical LNs can be processed to determine T cell cytokine production (Fig. 4) [18–20, 22, 23].

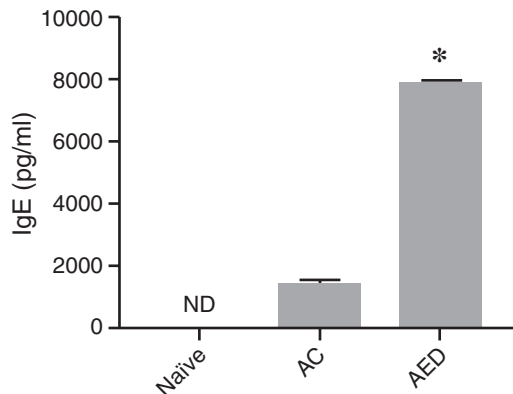


Fig. 3 AED results in higher IgE levels. Blood was collected on day 7, and serum IgE levels were assessed via ELISA per manufacturer's instructions. Data is representative of five independent experiments ($n = 5$ mice/group/experiment). $p < 0.05$ was considered significant

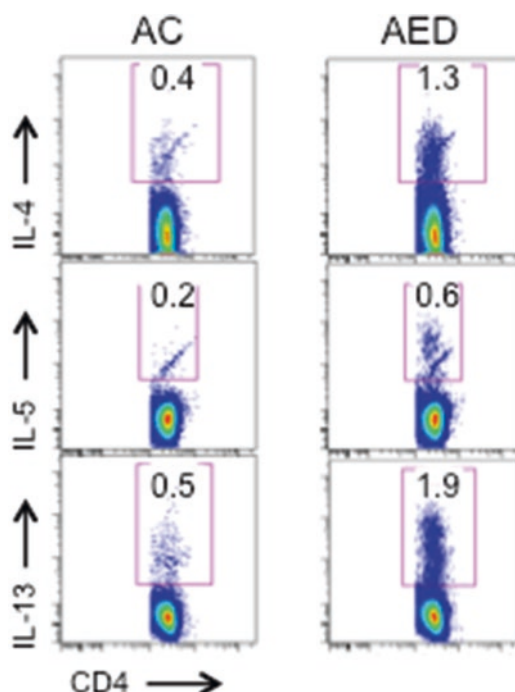


Fig. 4 *Th2 cytokine production was increased in AED compared to AC.* The LNs are collected after the last day of challenge and stimulated in vitro with PMA + I for 4 h. IL-4, IL-5, and IL-13 were all increased in mice with AED when compared to mice with AC and naïve controls. Data is representative of five independent experiments ($n = 5$ mice/group/experiment). $p < 0.05$ was considered significant

4 Notes

1. We recommend using female mice from Charles River Laboratories (Wilmington, MA) that are 7–10 weeks old. We have also used female mice from Jackson Laboratories (Bar Harbor, ME). Female mice tend to have a more robust inflammatory response at the clinical level, as compared to males. Also, adult mice show a greater response, as compared to younger mice (i.e., <7 weeks of age).
2. It is best to prepare a 10 mg/mL stock solution of OVA in PBS and add 1 μ L of the stock to the mix to give you a concentration of 10 μ g. Pertussis toxin can also be diluted as per manufacturer's instruction to 100 ng/ μ L stock, and 3 μ L of this stock can be added to mix.
3. It is also best to prepare double the volume for immunization, as a significant volume can be lost due to the viscous and sticky nature of the mixture.
4. Add the Inject Alum drop by drop using a micropipette for optimal mixing.
5. It is best to load only the 200 μ L needed per mouse as opposed to a large volume. This allows immunization suspension to be mixed in between mice.

6. The only difference between the two models in terms of the immunization mix is the absence of PT in the AC model.
7. For the slit-lamp examination, mice are not under anesthesia.
8. The 20-min time point is indicative of the early phase (i.e., immediate hypersensitivity response). It is primarily the result of IgE-dependent mast cell degranulation. By contrast, the 6-h time point is representative of the late-phase response in which there is cellular infiltration, primarily eosinophils (and other granulocytes) but also T cells.
9. Mice can be evaluated 24 h after topical challenge as an additional late-phase time point. However, the clinical response usually mirrors the 6-h time point.
10. Clinical signs should become apparent within 2 days of OVA application. If no clinical response is observed by day 3, then the immunization may not have been optimal. Make sure reagents have not expired.
11. The severity of the clinical disease and manifestations in the AED model can vary from experiment to experiment; however, it will consistently be more clinically severe than the AC model at the 6-h (and 24-h) time point. Additionally, in the AED model, there will also be increased leukocytic infiltration in the conjunctiva, higher serum and tear IgE levels, and higher Th2 frequencies in the LN compared to the AC model. Note, it is also helpful to include a naïve control with every experiment for comparison.
12. When testing topical interventions, it is important to include a vehicle control, as the vehicle can reduce the clinical response.
13. These severe clinical manifestations are present only in the AED model and do not develop in the AC model. They can be documented as being present or absent (severity is not scored on a scale). These manifestations typically peak between day 3 and day 4 of OVA administration.
14. Corneal epitheliopathy is confirmed by applying 1% fluorescein onto the cornea. The eye is then examined by slit-lamp microscopy using cobalt blue light. We assess this on day 6 after the 20-min time point.

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