Fc-epsilon receptor I of Primary Nociceptor as Detectors of IgE-Immunocomplex may Contribute to Itch in ocular allergy

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Abstract: Objective Pruritus is the major symptom of allergic conjunctivitis that affects up to 40% population in America while current treatments are often unsatisfying. Patients with allergic disorders usually have an elevated serum level of antigen-specific immunoglobulin E (IgE). Upon binding with antigen, IgE activates Fc-epsilon receptors (FcεR) on mast cells and other immune cells triggering a series of hypersensitivity reactions that may produce itch and/or pain. Previous reports suggested that the high-affinity activating IgG receptor (FcγRI) expresses on primary nociceptive neuron in the rat, and therefore it plays a role in the sensation of pain. In this study, we investigated the potential role of neuronal FcεRI in ocular pruritus. Methods Adult male C57BL/6 mice (20 to 25g; 6-8wk) were made allergic model of mice by injection of a solution of OVA plus Imject Alum. Topical OVA challenging to the eyes induced ocular allergy of mice. The mice were placed in an acrylic box (13×9×40 cm) for at least 1h to allow them for acclimation. They were returned to the same cells immediately after drops of the allergen or other chemicals, and their behavior was videotaped from the top of box with a high-resolution digital camera for 1h in the observation room without person. The numbers of bouts of scratching the treated eye with its hindpaw or wiping the treated eye with the ipsilateral forelimb were counted during video playback. qPCR and Western blotting were used to examine the expression level of FcεRIα, β and γ subunits, and IHC was used to check the cellular distribution of FcεRIα, β and γ subunits. We further examined whether neuronal FcεRI could be directly activated by IgE-IC using calcium imaging. Primary TG neurons were cultured, and IgE-IC was prepared by using the OVA as antigen and
the affinity-purified mouse anti-OVA IgE as antibody. **Results** (1) Scratching with the hindlimb as an indicator for ocular itch in the mice. When a pruritogen Bam8-22 was dropped to the eye, the mice showed significant increase of scratching but not wiping toward the eye; but after algogen capsaicin was dropped to the eye, the mice demonstrated a significant increase of wiping with the forelimb toward the eye rather than significant increase of scratching with the hindlimb. (2) Topical application of antigen (OVA, dropped to the eye) dose-dependently induced a pruritic behavior of the treated eye in allergic mice. And itch-specific scratching behaviors in the allergic mice were largely abolished by topical application of the blocking antibody to FcεRIα. (3) We found that three subunits of FcεRI (α, β and γ) expressed in subpopulations of trigeminal ganglion (TG) neurons in naïve mice, and FcεRIα and β were significantly upregulated in the allergic mice. (4) In dissociated TG neurons, IgE-immune complex, not the antibody or antigen alone, induced [Ca^{2+}] increase in the TG neurons via neuronal FcεRI. Both the amount of increase in R(340/380) and the responding rate of neurons induced by IgE-IC were significantly reduced in specific FcεRIα siRNA-transfected TG neurons. **Conclusion** Our findings suggest that primary sensory neurons including pruriceptors in the TG express functional FcεRI and could be directly activated by IgE-IC. Neuronal FcεRI, esp. FcεRIα may be increased in allergic mice with increased serum IgE, and may play an important role in ocular pruritus. Our findings reveal a novel mechanism of allergic pruritus in the eyes via direct activation of sensory neuronal FcεRI by IgE-IC. This study may suggest a novel strategy for the anti-pruritic treatment in allergic disease in the eyes as well as other diseases with pathological itch.

**Key words:** Fc-epsilon receptor type I; IgE immune complex; trigeminal ganglion; itch; nociceptive neurons