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Full-length Article

Neuronal Fc-epsilon receptor I contributes to antigen-evoked pruritus in a murine model of ocular allergy

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ABSTRACT

Pruritus is the major symptom of ocular allergy but currently available treatments are often ineffective. Previous studies demonstrated that subpopulations of primary sensory neurons express Fc receptors and may contribute to antigen-specific pain. We investigated the role of neuronal Fc-epsilon Receptor I (FcεRI) in allergic ocular pruritus. Ovalbumin (OVA) was used as allergen together with alum adjuvant (OVA + alum) to produce a mouse model of ocular allergy with a significant elevation in the serum levels of both antigen-specific IgE and IgG. Mice sensitized by OVA without alum only induced elevation of serum IgG but not IgE. Scratching behavior toward the eyes with the hindlimb was used as an indicator of ocular itch. Topical OVA challenging to the eye dose-dependently induced scratching toward the eye in the OVA + alum sensitized mice, but not those sensitized by OVA only. The antigen-induced scratching was largely abolished by topical application of the blocking antibody to FcεRIα, but was only partially alleviated by pretreatment of mast cell stabilizer or histamine I receptor antagonist. The expression of FcεRI was detected in subpopulations of trigeminal ganglion (TG) neurons including those expressing pruriceptive markers and innervating the conjunctiva in the naïve mice. Moreover, FcεRI was found significantly upregulated in small-sized TG neurons in the OVA + alum sensitized mice. In acutely dissociated TG neurons, IgE-immune complex (IC), but not the antibody or antigen alone, induced intracellular calcium increase. The neuronal responses to IgE-IC could be specifically blocked by pre-application of a siRNA for FcεRIα. Our results indicate that FcεRI expressed on peripheral nociceptive neurons in the TG may be directly activated by IgE-IC and contribute to allergic ocular pruritus. This study may suggest a novel mechanism for the development of pathological itch in allergic diseases.

1. Introduction

Itch is an unpleasant sensation that elicits the desire or reflex to scratch (Ikoma et al., 2006; Dhand and Aminoff, 2014). The sensation of itch shares a number of similar characteristics with pain and is likely transmitted by certain unmyelinated C fibers in the peripheral nerve that was classified as nociceptor or, more specifically, pruriceptors (Steinhoff et al., 2006; LaMotte et al., 2014). Antigen-specific immune diseases such as atopic and allergic dermatitis are often accompanied with pathological itch as well as pain (Galli and Tsai, 2012; Greaves and Wall, 1996). Patients with allergic disorders usually have an elevated level of antigen-specific immunoglobulin (Ig), especially IgE in the serum (Galli and Tsai, 2012). IgE may bind with specific allergen forming immune complex (IC). IgE-IC can induce itch via three steps: IgE-IC activate immune cells via Fc epsilon receptor (FcεR); activated immune cells release pro-inflammatory mediators and pruritogens (such as histamine, tryptase, cytokines); pruritogens induce itch by activating peripheral sensory neurons (Turner and Kinet, 1999; Rivera, 2002; Stone et al., 2010). It has been well known that IgE may activate certain immune cells such as mast cells upon binding with

Abbreviations: Bam8-22, bovine adrenal medulla peptide 822; DAPI, 4′, 6-diamidino-2-phenylindole; DRG, dorsal root ganglion; FcγRI, Fc-gamma-Receptor I; FcεRI, Fc-epsilon receptor I; FcεRII, Fc-epsilon receptor II; IC, immune complex; IgE, immunoglobulin E; IgG, immunoglobulin G; OVA, ovalbumin; TG, trigeminal ganglion.

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allergen and trigger a series of immune responses which can produce itch and/or pain. However, current therapeutics targeting histamine receptors or mast cells do not always effectively relieve the discomfort of itch or stop the destructive behavior which can produce tissue damage in certain pathological conditions such as allergic conjunctivitis or dermatitis.

FcεR type I (FcεRI) is the high-affinity activating receptor that binds to the Fc portion of IgE. FcεRI has been defined structurally as a trimeric form ($\alpha\gamma_2$) or a tetrameric form ($\alpha\beta\gamma_2$) (Turner and Kinet, 1999; Conner and Saini, 2005). The α-chain containing two immunoglobulin-type domains is the IgE binding chain. The β- and γ-chains contain conserved immunoreceptor tyrosine-based activation motifs (ITAMs) (Turner and Kinet, 1999; Rivera, 2002; Stone et al., 2010; Conner and Saini, 2005; Okayama et al., 2014). FcεRI typically expresses on immune cells (such as mast cell in skin and mucosa, or basophils in blood vessels). As suggested in a previous report (Andoh and Kuraishi, 2004; van der Kleij et al., 2010) and in our pilot studies (Liu et al., 2015, 2014), FcεRI may express in dissociated mouse dorsal root ganglia (DRG) neurons but the function of neuronal FcεRI was unknown. It was not clear whether the peripheral sensory neurons could be directly activated by IgE-IC.

We hypothesize that a subpopulation of peripheral nociceptive (including pruriceptive) neurons that express FcεRI can be directly activated by IgE-IC inducing itch and/or pain. Furthermore, the expression of neuronal FcεRI and responses to IgE-IC may be modulated in allergic diseases therefore contributing to the exaggerated pruritus in such conditions. We tested these hypotheses in ovalbumin allergic mouse model of ocular itch. Our findings revealed a novel neural mechanism for the sensation of itch, and might suggest potential therapeutic strategies for the treatment of pruritus related to allergic diseases.

2. Materials and methods

2.1. Animals

Adult male C57BL/6 mice (20–25 g; 6–8 wk, National Institutes for Food and Drug Control, Beijing, China) were used in this study. The *Mrgprd* (Zylka et al., 2005) and *Mrgpra3* (Han et al., 2013) transgenic mice were generous provided by Dr. Xinzhong Dong (Johns Hopkins University School of Medicine). Mice were housed in temperature (23 ± 3 °C) and 12 h light/dark cycle controlled rooms with standard rodent chow and water available. The experimental protocols were approved by the Institutional Animal Care and Use Committee in Chinese Academy of Medical Sciences, Institute of Basic Medical Sciences. Animals were randomly assigned to treatment or control groups.

2.2. Reagents

Capsaicin, BSA, BAM8-22, Cromolyn sodium, HEPES, ovalbumin (OVA), poly-D-lysine, laminin, terfenadine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Armenian hamster anti-FcεRIα antibody, Armenian Hamster monoclonal IgG Isotype control, and rabbit anti-FcεRIγ were from Abcam (Cambridge, UK). Rabbit anti-FcεRIα, mouse anti-FcεRIβ, and goat anti-FcεRIγ were from Santa Cruz Biotechnology (Dallas, Texas, USA). Guinea pig anti-PGP9.5 was from LifeSpan Biosciences (Seattle, WA, USA). Mouse anti-β-actin was from ZSJQ (Beijing, China). Alexa Fluor 488-conjugated donkey anti-rabbit, Alexa Fluor 488-conjugated donkey anti-mouse, Alexa Fluor 488-conjugated donkey anti-goat, and Alexa Fluor 594-conjugated donkey anti-guinea pig were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Protease inhibitors and chemiluminescent reagents (eECL Kit) were from CWBio (Beijing, China). Trypsin inhibitor, Liberase TL and Liberase TM were from Roche Diagnostics (Indianapolis, IN, USA). DMEM and F12 medium, FBS, Fura 2-acetoxy-methyl ester and Imject Alum, TriZol Reagent and Lipofectamine®RNAi MAX Reagent were from Thermo Fisher Scientific (Waltham, MA, USA). Papain was from Worthington Biochemical (Lakewood, NJ, USA). RT Master Mix and SYBR premix Ex TaqTM were from TAKARA (Dalian, China); VECTASHIELD Mounting Medium with DAPI was from Vector Lab (Burlingame, CA, USA).

IgE-IC was prepared by using the OVA as antigen and the affinity-purified mouse anti-OVA IgE (Acris Antibodies, Inc) as antibody. The storage buffer of all the antibodies (containing sodium azide as preservative) was exchanged to HEPES buffer using Zeba™ spin desalting columns (Thermo Scientific) before application to avoid the possible toxic or non-specific effects of sodium azide. IgE-IC were formed by incubating 1 μg/ml antigen and antibody at the ratio of 1:1 for 1 h at 25 °C, and then diluting to the concentrations of 0.01, 0.1, 0.3, and 1 μg/ml. In control experiments, individual components of IC (i.e. mouse anti-OVA IgE, OVA) were applied.

2.3. Sensitization and challenging of mice

A mouse model of ovalbumin sensitization induced ocular itch was produced with the following procedures: on day 0, day 7 and day 14, i.p. injection of 100 μg OVA plus 100 μl Imject Alum dissolved in 400 μl of normal saline. On days 21, 10 μl of OVA (0.001–5% in normal saline) were applied topically to both eyes to induce ocular allergy (Groneberg et al., 2003).

2.4. Behavioral assessment for ocular itch and pain

Behavioral assessment for ocular itch and pain in the mice were preformed following the procedures as described in previous publications (Shimada and LaMotte, 2008; Huang et al., 2016). Briefly, mice were placed in an acrylic box (13 × 9 × 40 cm) in a soundproof room without persons for 3 consecutive days before and 1 h on the day of recording to allow acclimation. They were returned to the same box immediately after drug administration, and were videotaped from the top of box with a high-resolution digital camera (SONY HANDYCAM HDR-PJ580E, Japan) for 1 h. Four mirrors were placed on the sides of the box for a better observation of the mouse behavior. The numbers of bout of scratching the treated eye with its hindpaw or wiping with the ipsilateral forelimb were counted during video playback.

2.5. Measurement of serum total and special IgG and IgE

The total IgG or IgE in the mouse serum were assessed using the mouse IgG or IgE ELISA kit (eBioscience), respectively. Briefly, Corning Costar 9018 ELISA plate was coated overnight at 4 °C with capture antibody. Coated wells were blocked with Blocking Buffer at room temperature. Samples were diluted at 10,000-fold for IgG or 25-fold for IgE. Total IgG was detected using HRP-conjugated anti-mouse IgG monoclonal antibody; total IgE was detected using biotin-conjugated anti-mouse IgE and Streptavidin-HRP monoclonal antibody as a second-step reagent, according to the manufacturer's instructions. Similarly, the OVA-specific IgG_1 or IgE were measured using a Mouse OVA IgG_1 or IgE ELISA kit (Cayman, Maine, USA), respectively.

2.6. Cell dissociation and culture

TG neurons were dissociated and cultured from adult C57BL/6 mice. Briefly, TGs of both sides were harvested from the skull base of decapitated mice after quick removal of brain, and transferred