Mouse Anti-Crude Peanut Extract IgE Monoclonal Antibody for Inducing and Studying Allergic Diseases

Catalog # 3069 and 3070

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INTRODUCTION

Type I hypersensitivity, which is characterized by an allergic reaction immediately following contact with innocuous antigens, is a typical clinical feature of allergic diseases such as asthma, eczema, hay fever, or urticaria. This hypersensitivity mediated by the IgE antibodies is called "atopic reagin" and the clinical features of type I hypersensitivity are described as "atopy". The high affinity receptor for IgE (FcεRI) expressed on mast cells and basophils is another critical component in allergic responses. IgE bound to FcεRI drastically up-regulates FcεRI expression on mast cells through stabilization and accumulation of FcεRI and contributes to further enhancing hypersensitivity responses to allergens (*1*). Specific allergens bound to IgE on cell surfaces will cross-link two FcεRIs and this linkage leads to the stimulation and degranulation of mast cells (*2*, *3*). This is associated with the release of a variety of proinflammatory mediators and cytokines such as histamine, proteolytic enzymes, heparin, and chemotactic factors, which cause the symptoms associated with type I hypersensitivity.

Mice are widely used experimental animals for studying pathogenesis and evaluating therapeutics in allergic disease models as a variety of inbred strains, transgenic mice, and even gene knockout mice are available (*2–6*). In order to induce allergic diseases in animals, immunize animals with an antigen adsorbed on aluminum hydroxide gel or expose animals to an aerosolized antigen repeatedly (*7–9*). In addition, IgE antibodies against specific antigens can sensitize animals by passive transfer *in vivo* as well as sensitize mast cells *in vitro* (*8–10*).

Among these allergens, the allergenicity of the peanut (whether it is raw or roasted) is the highest relative to other food allergens. Eleven potentially important peanut allergens have been identified. Of these, Ara h1, Ara h2, Ara h3, and Ara h6 have been designated the major peanut allergens. Ara h2 and Ara h6, two highly related 2S albumins, especially contribute to the development of allergic reactions (11).

Mouse peanut allergy models have been used to study the pathogenesis of the peanut allergy and to help develop new treatments. There are several protocols to develop mouse peanut allergy models such as 1) a combination of immunization with aluminum hydroxide adjuvant and oral administration (12,13), 2) oral administration alone (14-17), and 3) intranasal administration (18). Although these models are useful, developing allergic models takes 4-8 weeks because the immune system needs time to induce IgE antibodies against peanut antigens. In addition, serum IgE levels against allergens can vary significantly depending on the dose of the allergen, sensitization route, mouse strain, and protocol used. Inconsistent serum IgE levels among animals affects overall incidence and severity of allergic reactions in studies. To overcome these inherent flaws, Chondrex, Inc. developed mouse anti-crude peanut extract (CPE) IgE monoclonal antibodies and evaluated their specificities and biological activities with *in-vitro* and *in-vivo* studies. Chondrex, Inc. aims to establish an efficient allergic CPE mouse model by bypassing *in-vivo* IgE antibody development (29).

LIST OF CPE REAGENTS

Catalog #	Product	Amounts
3069	Crude Peanut Extract (CPE)	10 mg, Lyophilized
3070	Anti-CPE IgE Monoclonal Antibody, Clone 2G11G7	3 mg, Lyophilized

Chondrex, Inc. provides an IgE monoclonal antibody specific to CPE: clone 2G7G11. In general, the cross-linkage of IgE molecules bound to receptors on mast cells by a polyvalent allergen is required to trigger degranulation of mast cells. Alternatively, IgG or IgM antibodies specific to the allergen can trigger the activation of mast cells by cross-linking two allergens bound by IgE antibodies on the mast cell surfaces (10). 2G11G7 alone can induce degranulation of mast cells (RBL-2H3 cells) *in vitro* (Figure 1), and severe hypersensitivity *in vivo* (Figure 2) (29). Alternatively, Chondrex, Inc also provides a mouse IgE monoclonal antibody, isotype control, clone 2A101A12 (Cat # 7129) as a negative control antibody.

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EXPERIMENTAL PROTOCOLS

A. IMPORTANT: Handling the IgE Antibody Solution

IgE antibodies are biologically active at very low levels, so extra caution is required for handling and diluting IgE antibodies. In general, several µg of protein can adsorb to 1 square cm of glass or plastic surfaces by non-specific hydrophobic binding. Therefore, if 1 ml of a 20 µg/ml IgE antibody solution without blocking agents (such as PBS) is kept in a microcentrifuge tube, almost all the IgE antibodies will be adsorbed to the tube surface and no detectable IgE antibodies will remain in the solution. Do not dilute IgE solutions to less than 100 µg/ml with PBS as it does not contain blocking agents. Add a suitable blocking agent to PBS, such as mouse serum albumin, bovine serum albumin (BSA), or other suitable proteins. Chondrex, Inc. provides IgE Dilution Buffer (Cat # 3009) for diluting IgE antibodies for both *in vitro* and *in vivo* experiments.

B. Assay for Degranulating Mast Cells *In Vitro* (29)

1. Culture mast cells such as rat basophilic leukemia cells (RBL-2H3) until the stationary phase. Re-suspend the cells at 1 x 10^6 cells/ml in Eagle's minimum essential medium containing 15% fetal calf serum (FCS) and transfer cell suspension into 96-well (200 µl: 2 x 10^5 cell/well) or 24-well (1 ml: 1 x 10^6 cells/well) flat bottom cell culture plates and continue to culture at 37°C for at least 3 hours or overnight.

2. Add IgE monoclonal antibodies diluted with PBS containing blocking agents or culture medium containing FCS to the wells (final concentration: 0-10 µg/ml IgE antibodies in medium) and incubate for 24 hours.

   NOTE: Instead of adding IgE antibodies into culture media after overnight cell culture, IgE antibodies can be added to culture media at the start of cell culture. In this case, FcεRI expression could be significantly upregulated with the overnight culture. For example, it has been reported that FcεRI expression will be upregulated 2-fold in the RBL-2H3 cells and up to 32-fold on bone marrow-derived mast cells within 24-48 hours (*1*).

3. Wash IgE-sensitized cells 3 times with PBS

4. Add 200 µl (in 96-well plate) or 400 µl (in 24-well plate) of CPE solution diluted with Tyrode's buffer (150 mmol/l NaCl, 2.5 mmol/l KCl, 12 mmol/l $NaHCO_3$, 2 mmol/l $MgCl_2$, 2 mmol/l $CaCl_2$, 1 mg/ml BSA, 1 mg/ml dextrose, pH 7.4) and culture at 37°C for 1 hour (the final concentration of CPE: 2 µg/ml)

5. Add 200 µl (in 96-well plate) or 400 µl (in 24-well plate) of a negative control antigen such as BSA (2 µg/ml) dissolved in Tyrode's buffer into the negative control wells to determine spontaneous release of β-hexosaminase.

6. Add 200 µl (in 96-well plate) or 400 µl (in 24-well plate) of 1% Triton X-100 in Tyrode's solution into 100% control wells to determine the total release of β-hexosaminase.

7. Assay β-hexosaminase activity in the culture supernatant using p-nitro-phenyl-N-acetyl b-D-glucosaminide as a substrate (19).

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Figure 1. Degranulation of RBL-2H3 cells by CPE and anti-CPE IgE monoclonal antibodies

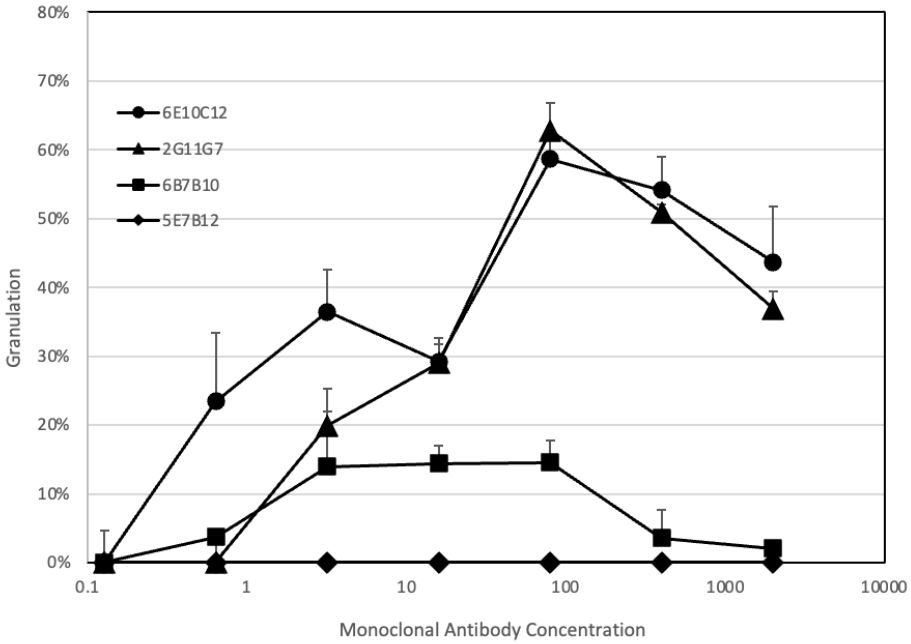

Figure 1. RBL-2H3 cells were cultured in 1 ml of DMEM containing 15% FBS at 10^6 cells/well in a 96-well plate for 3 hours at 37 degrees C and then treated with anti-CPE IgE monoclonal antibodies, 6E10C12 (Circle), 2G11G7 (Triangle), 6B7B10 (Square), and 5E7B12 (Diamond), at 37 degrees C for 16 hours. After washing cells with PBS two times, 2 µg/ml of CPE in Tyrode's buffer at 200 µl/well were incubated at 37 degrees C for 1 hour. 100 µl of each sample was used for assaying β-hexosaminase activity. The degranulation of RBL-2H3 cells were expressed as a ratio compared with 100% degranulation of the cells which received 1% Triton-X in Tyrode's buffer. 2G11G7 and 6E10C12 showed degranulation with a bell-shaped dose–response curve. The degranulation reached 60% at 100 ng/ml. Meanwhile, 6B7B10 showed lower activation levels and 5E7B12 did not react at all.

C. Assay for Type I Hypersensitivity Reactions *In Vivo (29)*

Inject 100 - 300 µl of the diluted IgE monoclonal antibody solution intravenously into mice (10 - 1000 µg/mouse). Inject 25 µl of allergen (2 mg/ml CPE in PBS) intradermally at the foot pad (50 µg/foot pad) after 24 hours. Determine the swelling of the footpad by measuring the footpad thickness using a dial thickness gauge every 1-2 hours for 24 hours.

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Figure 2. Footpad Delayed Hypersensitivity

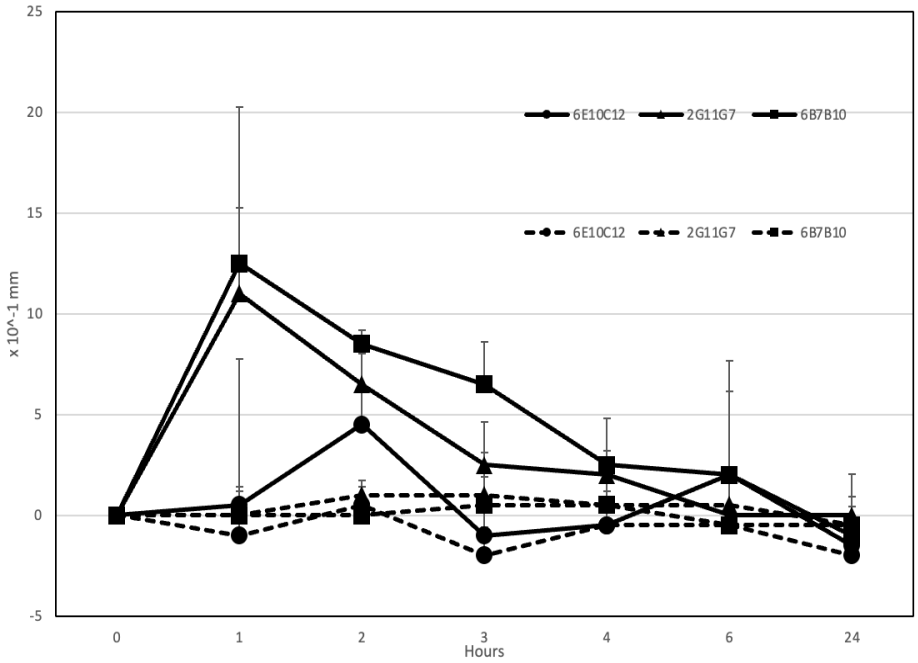

Figure 2. Footpad thickness of mice after intravenous administration of 1 mg of monoclonal antibodies: 6E10C12 (Circle), 2G11G7 (Triangle), 6B7B10 (Square), followed by 50 µg of CPE (Solid line) or PBS (Dashed line) intradermal injection at footpad. Female 7-8 week old BALB/c mice were intravenously injected with 330 µl of IgE antibody solution (3 mg/ml), then injected with 25 µl of CPE (2 mg/ml in PBS) intradermally at the foot pad (50 µg/foot pad) after 24 hours. IgE mAbs 2B11G7 and 6B7B10 induced paw swelling, 1.10 +/- 0.42 mm and 1.25 +/- 0.78 mm, respectively that peaked at 1-2 hours after the CPE injection at the footpad and resolved to base levels within 6 hours. However, 6E10B10 failed to induce paw swelling.

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