

Mouse Monoclonal IgE Antibodies Against Ovalbumin for Studying Allergic Diseases in Mice

Catalog # 3006-3008

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INTRODUCTION

Type I hypersensitivity, which is characterized by an allergic reaction immediately following contact of innocuous antigens, is a typical clinical feature of allergic diseases, such as asthma, eczema, hay fever, and urticaria. This hypersensitivity is mediated by IgE so 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 (1) and contributes in further enhancing hypersensitivity responses to allergens. Specific allergens bound to IgE on the cell surfaces forms cross-linkage of FcεRI (2 - 3), which leads to the stimulation and degranulation of mast cells. 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 have been widely used for experimental models of allergic diseases such as asthma for studying pathogenesis (2 - 4) and evaluating therapeutics (5, 6), since a variety of inbred strains, transgenic mice, and even gene knockout mice are available. 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). However, it is well known that IgE antibody against specific antigen can sensitize animals by passive transfer in vivo (8, 9) as well as sensitize mast cells in vitro (10). For example, passive transfer of airway hyper-responsiveness by ovalbumin (OVA)-specific monoclonal IgE and IgG antibodies was reported by Oshiba et al (8), although eosinophilic inflammatory response in the airway is modest despite high levels of serum IgE (8).

LIST OF IgE AND IgG MONOCLONAL ANTIBODIES AGAINST OVA

Chondrex provides two IgE monoclonal antibodies specific to OVA, clone E-C1 and clone E-G6 (13, 14), in addition to one IgG1 monoclonal antibody against OVA, clone L71 (see Type I Hypersensitivity Research Products on page 2). In general, the cross-linkage of IgE molecules bound to the receptor on mast cells by a polyvalent allergen is required to trigger degranulation of mast cells. Alternatively, IgG antibody to the allergen can trigger the activation of mast cells by cross-linking of two antigens bound by IgE antibody on the mast cell surfaces (10). However, E-C1 alone is capable of inducing degranulation of mast cells in vitro (Figure 1), and severe hypersensitivity reaction in vivo (Figure 2). Therefore, it is assumed that E-C1 might recognize repetitive epitopes of OVA. On the other hand, E-G5 is not capable of inducing these hypersensitivity reactions by itself in vitro and in vivo (Figures 1 and 2), and can be used as control. Furthermore, it has been reported that aggregated OVA, which carries multiple epitopes, increases the formation of cross-linkage of IgE on mast cell surfaces (15).

IgG1 antibody, L71, can be used for variety of research purposes such as studying the interference between allergen specific IgG and IgE antibodies, since pathogenic roles of IgG in allergic diseases remains uncertain (11). For example, IgG antibody might compete with IgE antibody and block the immediate hypersensitivity reactions or IgG might stimulate IgE-mediated hypersensitivity reactions by cross-linking of allergen bound to IgE on the surfaces of mast cells or IgG-antigen immune complex might stimulate IgE mediated hypersensitivity reactions by forming multivalent antigen which might bind to IgE on the surfaces of mast cells.

TYPE I HYPERSENSITIVITY RESEARCH PRODUCTS

Catalog #	Description	Antigen	In Vitro Mast Cell Activation	In Vitro Hypersensitivity Reaction	Quantity	Storage
3006	Mouse Monoclonal IgE Antibody E-C1	OVA & Aggregated OVA *	Yes	Yes	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C
3007	Mouse Monoclonal IgE Antibody E-G5	OVA Only **	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C
3008	Mouse Monoclonal IgG1 Antibody L71	OVA & Aggregated OVA	No	No	1 mg/vial Lyophilized with Mouse Serum Albumin	-20°C

* Aggregated OVA can be prepared by mixing 2 ml of OVA solution (20 mg/ml in PBS, pH 6.0) and 1 ml of 2.4% of glutaraldehyde diluted with PBS (pH 6.0) at room temperature overnight. Add 1 ml of 1M glycine to neutralize excess amounts of glutaraldehyde and react at room temperature for 30 minutes, and then dialyze against PBS, pH 7.2. In order to remove un-aggregated OVA, run Sephacryl S-400 gel filtration chromatography (15).

** Does not react with glutaraldehyde-aggregated OVA.

EXPERIMENTAL PROTOCOLS

1. Handling of IgE antibody solution

Since IgE antibody is biologically active at very low levels, extra caution is required to handle this material. In general, several 10 µg of protein will be adsorbed on 1 square cm glass and plastic surfaces by non-specific hydrophobic binding. Therefore, if 1 ml of 20 µg /ml of IgE solution is kept in a tube, almost all of IgE will be adsorbed and no detectable IgE will remain in the solution. Do not dilute IgE solution to less than 100 µg/ml with PBS which 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. IgE Dilution Buffer (catalog # 3009) is preferred for diluting IgE for both in vitro and in vivo experiments.

2. Assay for degranulation of mast cells in vitro

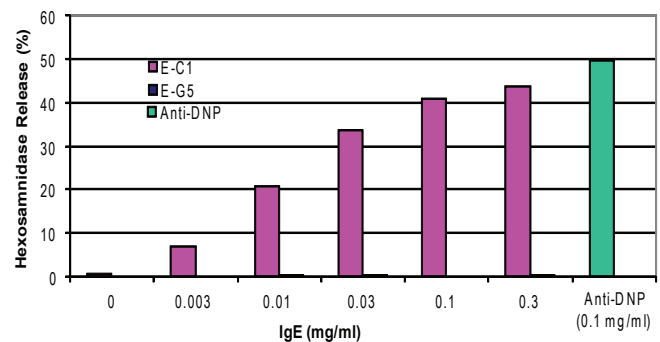
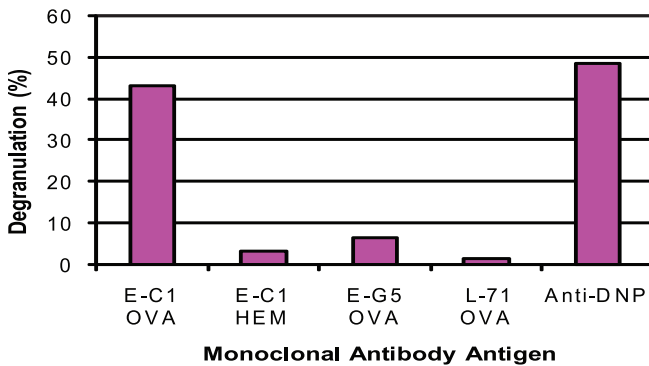
1. Culture mast cells such as rat basophilic leukemia (RBL-2H3) until the stationary phase. Re-suspend the cells at 2.5×10^5 cells/ml in Eagle's minimum essential medium containing 5% fetal calf serum (FCS), and transfer cells into 96-well (250 µl: 6.25×10^4 cell/well) or 24-well (1 ml: 2.5×10^5 cells/well) flat bottom cell culture plate, and continue to culture at 37°C overnight.

- Add IgE monoclonal antibody diluted with PBS containing blocking agents or culture medium containing FCS to the wells (final concentration: 0-10 $\mu\text{g/ml}$ of medium) and incubate for 1 hour.

Note: Instead of adding IgE into culture medium after overnight cell culture, IgE can be added to culture medium at the start of cell culture. In this case, Fc ϵ RI expression could be significantly up-regulated within overnight culture. For example, it has been reported that Fc ϵ RI expression will be up-regulated 2 fold on RBL cell lines and up to 32 fold on bone marrow-derived mast cells within 24-48 hours (1).

- Wash IgE-sensitized cells 3 times with Tyrode's buffer (150 mmol/L NaCl, 2.5 mmol/L KCl, 12 mmol/L NaHCO₃, 2 mmol/L MgCl₂, 2 mmol/L CaCl₂, 1 mg/ml BSA, 1 mg/ml dextrose, pH 7.4).
- Add 200 μl (in 96-well plate) or 400 μl (in 24-well plate) of OVA solution diluted with Tyrode's buffer (the final concentration of antigen: 0-5 $\mu\text{g/ml}$) and culture for 1 hour.
- Add 200 μl (in 96-well plate) or 400 μl (in 24-well plate) of a negative control antigen such as hen egg lysozyme (HEL) or BSA (5 $\mu\text{g/ml}$) dissolved in Tyrode's buffer into the negative control wells to determine spontaneous release of β -hexosaminase.
- Add 200 μl (in 96-well plate) or 400 μl (in 24-well plate) of 1% Triton X-100 solution dissolved in Tyrode's solution into 100% control wells to determine the total release of β -hexosaminase.
- Assay β -hexosaminase activity in the culture supernatant using p-nitro-phenyl-N-acetyl b-D-glucosaminide as a substrate (11).

Figure 1 - Degranulation of mast cells by monoclonal IgE antibody: a) comparison of 3 monoclonal antibodies and b) Dose response effect of E-C1 and E-G5



- a) Rat Basophil Leukemia Cells cultured in 24-well plate were sensitized by monoclonal antibody, E-C1 (1 $\mu\text{g/ml}$), E-G5 (1 $\mu\text{g/ml}$) and L71 (50 $\mu\text{g/ml}$), respectively, and then activated by OVA (5 $\mu\text{g/ml}$). As positive control, a combination of anti-DNP IgE antibody (1:200 dilution of culture media) and DNP-HSA (0.05 $\mu\text{g/ml}$) was used. E-C1 alone is capable of activating mast cells in antigen-specific manner, whereas E-G5 (IgE) and L-71 (IgG1) are not capable under the same condition.

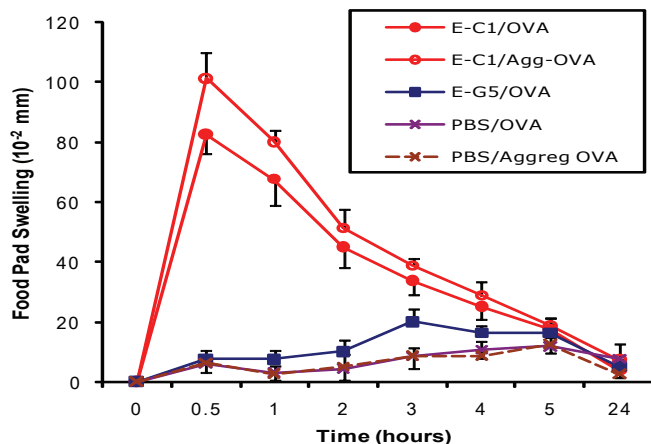
- b) Rat Basophil Leukemia Cells cultured in 24-well plate were sensitized with E-C1 and E-G5 (0-0.3 $\mu\text{g/ml}$), respectively and activated by OVA (5 $\mu\text{g/ml}$). As a positive control, cells were sensitized by anti-DNP IgE antibody (1:200 dilution of culture medium) and activated by DNP-HSA (0.05 $\mu\text{g/ml}$).

Note: Aggregated OVA causes an equal degree of degranulation of E-C1-sensitized mast cell at 1/5 concentration of OVA (data not shown).

3. Assay for hypersensitivity reaction in vivo

Dilute IgE antibody solution (1 mg/ml) to 100 μ g/ml with PBS containing a blocking agent such as mouse serum albumin. Inject 100 μ l of the diluted IgE monoclonal antibody solution to mice (10 μ g/mouse) by IV. Inject 25 μ l of OVA (2 mg/ml in PBS) intradermally at the food pad (50 μ g/foot pad) after appropriate period. Determine the swelling of footpad by measuring the footpad thickness using dial thickness gauge every 1-2 hours for 24 hours.

Figure 2 - Hypersensitive reaction at food pad in vivo



Bal/c mice received 10 μ g of monoclonal antibody, E-C1 or E-G5 by IV injection, then challenged by OVA (50 μ g) or aggregated OVA (Agg-OVA, 50 μ g) by ID injection 24 hours later. Food pad thickness was determined by a Loop Handle Dial Thickness Gauge, and shown as mm.

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