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Blocking Allergic Reaction through Targeting Surface-Bound IgE with Low-Affinity Anti-IgE Antibodies

Ke Zhang,^{*,†} Jeffrey Liu,^{*} Thao Truong,[†] Elyssa Zukin,[†] Wendy Chen,[†] and Andrew Saxon^{*,†}

Allergic disorders have now become a major worldwide public health issue, but the effective treatment options remain limited. We report a novel approach to block allergic reactivity by targeting the surface-bound IgE of the allergic effector cells via low-affinity anti-human IgE Abs with dissociation constants in the 10^{-6} to 10^{-8} M range. We demonstrated that these low-affinity anti-IgE mAbs bind to the cell surface-bound IgE without triggering anaphylactic degranulation even at high concentration, albeit they would weakly upregulate CD203c expression on basophils. This is in contrast to the high-affinity anti-IgE mAbs that trigger anaphylactic degranulation at low concentration. Instead, the low-affinity anti-IgE mAbs profoundly block human peanut- and cat-allergic IgE-mediated basophil CD63 induction indicative of anaphylactic degranulation; suppress peanut-, cat-, and dansyl-specific IgE-mediated passive cutaneous anaphylaxis; and attenuate dansyl IgE-mediated systemic anaphylaxis in human FcεRIα transgenic mouse model. Mechanistic studies reveal that the ability of allergic reaction blockade by the low-affinity anti-IgE mAbs was correlated with their capacity to downregulate the surface IgE and FcεRI level on human basophils and the human FcεRIα transgenic mouse bone marrow-derived mast cells via driving internalization of the IgE/FcεRI complex. Our studies demonstrate that targeting surface-bound IgE with low-affinity anti-IgE Abs is capable of suppressing allergic reactivity while displaying an excellent safety profile, indicating that use of low-affinity anti-IgE mAbs holds promise as a novel therapeutic approach for IgE-mediated allergic diseases. *The Journal of Immunology*, 2017, 198: 000–000.

Immunoglobulin E-mediated allergic diseases, including asthma and severe food allergy, have significantly increased in both prevalence and incidence over the past decades in the United States and other developed countries to the point where they now have become a major worldwide public health issue (1, 2). Currently, ~40% of the U.S. population is sensitized to IgE of one or more common inhalant or food allergen, and half of those will be symptomatic at any one time (3, 4).

Recognition of this epidemic of IgE-driven allergy disease and asthma has led to a corresponding interest in the development of novel immune-based biologic therapies. Although major efforts are being undertaken to develop better therapeutic approaches for these

allergic disorders, new effective and safe therapies for prevention/treatment of severe allergic disorders remain quite limited. Omalizumab (Xolair)-based anti-IgE therapy that binds free but not FcεRI-bound IgE was the only approved biologic for allergy disease and asthma for nearly 13 y; has shown modest success in reducing allergic airway reactivity in a subset of asthmatics (5, 6); and recently has been approved for chronic urticaria. Very recently, anti-IL-5 was approved for a subset of asthma (7), whereas a variety of other novel therapeutic approaches are in various stages of development. Thus, the effective treatment of severe allergic disorders continues to be a major unmet medical need.

Direct targeting of the IgE bound to FcεRI on basophils/mast cells using anti-IgE Abs has not been considered a feasible therapeutic approach due to the expectation that anti-IgE Abs would very likely trigger immediate degranulation by cross-linking the surface IgE/FcεRI complexes when the administered doses escalate to a certain point. However, it was also discovered that some anti-IgE and anti-FcεRI Abs could induce allergic effector cell desensitization, but not allergic degranulation (8); the naturally occurred anti-IgE autoantibodies from normal and allergic subjects were able to inhibit allergen-induced basophil activation (9); and the anti-IgE and anti-FcεRIα autoantibodies in chronic urticaria patients could render basophils unresponsive to following anti-IgE stimulation (10). These findings suggest that anti-IgE Abs do not necessarily induce degranulation; instead, some of them could induce allergic desensitization, just as that of a very low dose of allergen administration in the course of allergen immunotherapy. The exploit of those anti-IgE Abs with the allergic desensitization capacity as a novel allergy therapy has not been attempted previously. In this work, we report a novel approach using low-affinity anti-IgE (LAIGE) mAbs that target surface-bound IgE on allergic effector cells to suppress allergic reactions. This work demonstrates that LAIGE Abs have potential for therapeutic use for IgE-driven allergic disorders while showing a good safety profile.

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Abbreviations used in this article: BAT, basophil activation test; BMMC, bone marrow-derived mast cell; EBD, Evans blue dye; hFcεRIα Tg, human FcεRIα transgenic; LAIGE, low-affinity anti-IgE; mIgG1, mouse IgG1; PAE, polyclonal anti-IgE Ab; PCA, passive cutaneous anaphylaxis; ppIgE, paraprotein IgE.

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Materials and Methods

Abs and reagents

FITC- and PE-labeled anti-human CD123 (clone 6H6), CD63 (clone H5C6), PerCP-HLA-DR (clone L243), PE- and Alexa 647-labeled or unlabeled anti-human FcεRIα (clone AER-37), PE-CD203c and allophycocyanin-CD203c (clone NP4D6), and anti-mouse CD117 (c-kit) (clone 2B8) were purchased from BioLegend (San Diego, CA). Polyclonal anti-human IgE Ab (PAE) derived from goat (Ab9159) was purchased from Abcam (Cambridge, MA). Alkaline phosphatase-labeled anti-human IgM, IgG, IgA, and IgE, and anti-mouse IgG were obtained from KPL (Gaithersburg, MD); mouse rIL-3 from PeproTech (Rocky Hill, NJ); and the natural cat allergen Fel d1, peanut allergen Ara h1, Ara h2, and Ara h6 from Indoor Biotechnologies (Charlottesville, VA). The prescreened peanut- and cat-allergic plasmas were purchased from PlasmaLab International (Everett, WA). Anti-human IgE mAbs were produced with standard hybridoma technology (11) and purified with protein A and protein L (GenScript, Piscataway, NJ) affinity chromatography.

Surface plasmon resonance studies

The affinity determination of the anti-IgE mAbs was performed on a Biacore T2000 instrument (Biacore AB, Uppsala, Sweden). Human myeloma IgE (paraprotein IgE [ppIgE] and PSiGE) was immobilized on the CM5 sensor chips by amine coupling. The purified anti-IgE mAbs were dissolved in HBS-EP assay buffer containing 0.15 M NaCl, 10 mM HEPES (pH 7.4), 3 mM EDTA, and 0.005% polysorbate 20. The solutions traversed the sensors at a flow rate of 50 μl/min for binding analysis. Binding results were expressed in resonance units. Kinetic studies were analyzed with BIAevaluation software version 4.1, and the calculated affinity results fitted well from the two channels coupled with ppIgE and PSiGE.

Allergic subject population

A total of 23 (15 male, 8 female) healthy and 13 previously clinically diagnosed peanut (5 male and 2 female)- and cat (3 male and 3 female)-allergic subjects was recruited for this study. Allergic subjects' allergic status was confirmed by basophil activation test (BAT). Usage of human blood for BAT from allergic subjects was approved by the University of California, Los Angeles, Institutional Review Boards Committee.

Purification of peanut- and cat-allergic IgE

Pooled peanut- and cat-allergic donor plasmas were diluted 1:1 with PBS and passed through an anti-human IgE mAb (clone CIA-E4.15) (12)-coupled Sepharose 4B resin at the flow rate of 1 ml/min. The affinity column was washed with PBS, and the bound IgE fraction was eluted with elution buffer (10 mM glycine-HCl [pH 2.5]). The eluted fraction was combined and dialyzed against PBS overnight, followed by ELISA determination of the total IgE concentration.

Multiplex luminex assay

The human ppIgE-sensitized human FcεRIα transgenic (hFcεRIα Tg) mouse serum samples collected at 30 min post-anti-IgE mAb challenge were used to measure the allergic mediator release with multiplex luminex assay (Mouse Cytokine/Chemokine Magnetic Bead Panel) for mouse mast cell protease-1, eotaxin, IL-4, IL-5, IL-10, and IL-13. This assay service was provided by Westcoast Biosciences (San Diego, CA).

Basophil activation test

To determine the triggering capacity of allergen or anti-IgE Abs, BAT was performed using heparinized whole blood (100 μl/test) within 4 h of blood draw, with the stimulating time at 15 min, 37°C, for peanut or cat allergens, and 30 min for anti-IgE mAbs E4.15, E7.12, C6, E5.1, p6.2, and E2.18. For therapeutic effect determination using BAT-based approach, the allergic (peanut and cat) subjects' heparinized whole blood (200 μl/test) was incubated with mouse anti-human IgE mAbs or mouse IgG1 (mIgG1) isotype control in the ranges of 0.01–0.1 μg/ml for the high-affinity anti-IgE mAb E4.15, E7.12, C6, and E5.1, and the range of 0.25–4 μg/ml for low-affinity anti-IgE mAb p6.2 and E2.18. The tested blood was incubated with anti-IgE mAbs at room temperature (22–24°C) with slow agitation (100 rpm) for 24 or 48 h prior to basophil activation by the mixed purified Ara h allergens (Ara h1, h2, and h6 combined) or cat allergen Fel d1, respectively, for 15 min at 37°C with shaking. The activation was stopped by EDTA at final concentration of 10 μM. The allergen and anti-IgE Ab-stimulated blood samples were stained with CD123-FITC, CD63-PE, HLA-DR-PerCP, and CD203c-allophycocyanin, followed by erythrocyte lysis and flow cytometry acquisition and analysis (13–15). The cut-off value of the unstimulated blood basophil CD63 expression was always set as <3% as the background,

and CD203c expression level <10% because unstimulated basophils constitutively express low-level CD203c. Histamine release of basophils was performed with the ELISA kit from Immuno-biological Laboratories (IBL-America, Minneapolis, MN), following the instruction manual.

Human IL-4 production from basophils

PBMCs of nonallergic donors (~5 × 10⁸ cells from each donor) were used for enrichment of basophils with human basophil enrichment kit from StemCell Technologies (Cambridge, MA), following the kit instruction. The basophil purity varied from 54 to 78% measured by flow cytometry staining with CD123-FITC/FcεRIα-PE. The enriched basophils were cultured at the density of 5 × 10⁵ cells/ml in complete RPMI 1640 stimulated with isotype control (mIgG1 at 20 μg/ml); LAIGE p6.2 at 0.2, 2, and 20 μg/ml; and PAE at 0.1 μg/ml, respectively, for 1, 2, and 4 h. IL-4 levels in the culture supernatants were measured with human IL-4 ELISA kit from MyBioSource (San Diego, CA).

Determination of the allergic therapeutic index with BAT-based method

The dose of the anti-IgE mAbs sufficient to block 50% of Ara h allergen-induced CD63 expression with BAT was arbitrarily defined as the BAT effective therapeutic dose, whereas the lowest concentration of the anti-IgE mAbs capable of inducing >5% CD63 expression was arbitrarily defined as the BAT triggering dose. The BAT-based therapeutic index was calculated as the triggering dose divided by the effectively therapeutic dose. The larger the therapeutic index, the safer the anti-IgE mAb is predicted to be as therapy for IgE-mediated allergic diseases.

Passive cutaneous anaphylaxis

The previously established cat-, peanut-, and dansyl-specific IgE-mediated PCA assays were used to assess the ability of LAIGE mAbs to suppress cutaneous IgE-mediated allergic reactivity in hFcεRIα Tg mice (16). The hFcεRI Tg mice (17) were sensitized with the purified allergic IgE and the recombinant dansyl-specific IgE (18) at 2, 1, 0.5, and 0.125 μg/ml through intradermal injection on the back skin for 2 h, followed by injecting the mouse i.p. with the testing LAIGE mAbs (2 μg/g body weight), with the same amount of mIgG1 as an isotype control. Two and 4 d later, the animals were challenged i.v. with Fel d1 (10 μg); Ara h1, 2, and 6 combined (10 μg); or dansyl-BSA (100 μg) mixed with 100 μl of 2% Evans blue dye (EBD) dissolved in saline. The mice were euthanized 30 min after allergen challenge for assessing PCA reaction by photography. The mouse skin was dried overnight, the injected skin areas were cut out and weighed, and EBD was extracted with formamide at 55°C overnight. The extravasated EBD was quantified with a spectrophotometer at the wavelength of either 620 (19) or 650 nm with a conventional microtiter ELISA reader. The quantitative results from both methods fit well. EBD quantity from each PCA spot was normalized as per milligram skin tissue. The use of hFcεRIα Tg mice was approved by University of California, Los Angeles, animal research committee.

Systemic anaphylaxis assay

The hFcεRIα Tg mice were sensitized with a total of 40 μg (20 μg twice at 16-h interval) of recombinant dansyl-specific IgE (18) overnight (~24 h), followed by injection with p6.2 (50 μg per mouse) or mIgG1 isotype as control. Four days later, the mice were i.v. challenged with dansyl-BSA (100 μg per mouse) to induce systemic anaphylaxis. Core body temperature changes and the anaphylaxis clinical index (scores) were measured as indicators of systemic allergic reactivity. The anaphylaxis clinical score was defined as 0, no symptoms; 1, scratching and rubbing around the nose and head; 2, puffiness around the eyes and mouth, pilar erecti, diarrhea, and reduced activity or standing still with an increased respiratory rate; 3, wheezing, labored respiration, and cyanosis around the mouth; 4, symptoms as in number 3 with loss of consciousness, tremors, and/or convulsion; and 5, death (20). The core body temperature changes were monitored every 5 min for the first hour after dansyl-BSA challenge. The temperature changes were plotted for comparison and statistical analysis to determine the effect of p6.2 and control mIgG1 on systemic anaphylaxis (21).

Bone marrow-derived mast cells from hFcεRIα Tg mouse

The bone marrow was flushed out from the femurs and tibias of the hFcεRIα Tg mouse with 0.22 μm filtered flushing medium (DMEM supplemented with 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 5 × 10⁻⁵ M 2-ME). The collected bone marrow cells were cultured in two T75 flasks with 10 ml culture medium (flushing medium plus 10 ng/ml mouse rIL-3) each. Three days later, the suspended cells from each flask were transferred and expanded into two flasks with fresh culture medium. One week later, the suspended cells from each flask were expanded into two to three flasks, maintaining the cell density in ~1 ×

10^6 cells/ml. Half of the culture medium was replaced with fresh medium every 3–4 d, and the suspended cells were transferred to new flasks until no adhere cells were observed. Six weeks later, the suspended cells were stained with anti-mouse CD117-FITC and anti-human hFcεRIα-PE to assess the maturity of the cultured bone marrow–derived mast cells (BMMCs).

Confocal microscopy

BMMCs were centrifuged at 1500 rpm for 3 min; the supernatant was carefully aspirated; the cell pellets were washed with $2\times$ PBS, followed by blocking with 10% of normal mouse serum, and incubated for 30 min on ice; and then diluted FITC-labeled myeloma IgE (PSIgE) was added at 5 $\mu\text{g}/\text{ml}$ for sensitization for 2 h. After being washed, the FITC-IgE–sensitized BMMCs (1×10^6 cells/ml) were incubated with the control mIgG1 and p6.2 at 2 $\mu\text{g}/\text{ml}$, respectively, for 24 or 48 h at 37°C. The BMMCs were fixed with 0.5 ml of 2% formaldehyde (Ultrapure, electron microscopy grade 10% solution, dilute to 2% in PBS; Polysciences) for 30 min. After completing the above staining procedure, the tube(s) was kept at room temperature (dark) for 20 min. The cells were washed with PBS twice, followed by adding 50 μl of permeabilization medium, along with the Alexa 647–labeled anti-human FcεRIα for incubation at room temperature for 30 min. The washed cells were then fixed with 0.5 ml of 2% formaldehyde and spanned to the poly-lysine–coated glass slide using cytospin at 500 rpm for 5 min. The cells on the slides were stained with a drop of ProLong Gold anti-fade reagent with DAPI (Life Technologies, Eugene, OR). The slides were examined with Leica SP2-1P-FCS confocal microscope.

Statistics

Data are expressed as mean $\pm$ SEM. Unless indicated in the figure legends, Student *t* test was used to determine the statistical significance for the paired data. Statistically significant differences are defined as $*p < 0.05$ and $**p < 0.01$ in the appropriate figures.

Results

Comparison of basophil CD63 expression induced by allergens and high- and low-affinity anti-IgE mAbs

Anti-IgE Abs are expected to engage the cell surface–bound IgE in a fashion similar to allergen-mediated cross-linking of the surface FcεRIs on basophils and mast cells, leading to anaphylactic degranulation and resulting in allergic reaction. However, some anti-IgE Abs and autoantibodies, rather than triggering allergic degranulation, induced allergic effector cell desensitization and blocked allergen-induced basophil activation through currently unknown mechanism (8, 9). These studies suggested that not all anti-IgE Abs would trigger allergic degranulation; instead, some anti-IgE Abs would render allergic effector cells desensitized. Such anti-IgE Abs with the ability to desensitize allergic effector cells might have the potential to function as a novel therapeutic for IgE-mediated allergic diseases. To investigate the reason that various anti-IgE Abs display differential ability for allergic triggering, we first defined the ability of an array of anti-human IgE mAbs to induce basophil activation employing BAT with peanut- and cat-allergic subjects' blood challenging with relevant allergens.

BAT profiles of peanut ($n = 3$)- and cat ($n = 2$)-allergic subjects' blood were shown in Fig. 1, in which basophil surface CD63 expression, a marker of basophil activation indicative of anaphylactic degranulation (13–15), was induced by the peanut allergen Ara h2 (Fig. 1A–C) and cat allergen Fel d1 (Fig. 1D, 1E) in the dose range of 0.002–0.2 $\mu\text{g}/\text{ml}$. A high-affinity anti-IgE mAb E4.15, with K_d at 9.56×10^{-10} M, also triggered significant basophil CD63 expression in the dose range between 0.2 and 20 $\mu\text{g}/\text{ml}$ (Fig. 1A–E, middle rows). A LAIGE mAb p6.2 ($K_d = 2.54 \times 10^{-6}$ M), which binds to a conformational epitope on IgE CH2–CH4 region with low affinity (Supplemental Fig. 1), did not induce CD63 expression at 0.2–20 $\mu\text{g}/\text{ml}$ (Fig. 1A–E, lower rows) or even up to 100 $\mu\text{g}/\text{ml}$. A quantitative comparison of CD63 expression induced by all of the stimuli at various concentrations from these allergic subjects is summarized in Fig. 1F. These data indicate that both allergens and the high-affinity anti-IgE E4.15

are capable of activating basophils, with the activation potency of E4.15 being 2–3 logs lower than that of Ara h2 or Fel d1 allergens to achieve similar level of basophil activation. In contrast, the LAIGE p6.2 did not induce basophil CD63 expression even at high concentrations in these peanut- and cat-allergic donors, indicating that LAIGE p6.2 did not trigger basophil anaphylactic degranulation from both peanut- and cat-allergic subjects.

Comparison of basophil CD203c expression promoted by allergens, high-affinity anti-IgE, and LAIGE mAbs

Although not directly linked with anaphylactic degranulation (13, 22, 23), CD203c upregulation on basophils is also frequently used as an alternative basophil activation marker (24, 25). To test whether LAIGE p6.2 promoted CD203c expression, we simultaneously assessed CD203c and CD63 expression on basophils induced by cat allergen Fel d1, PAE, high-affinity anti-IgE mAb E4.15, and LAIGE p6.2. In cat-allergic donors ($n = 3$), the allergen Fel d1 and E4.15 induced robust basophil CD203c and CD63 expression, with apparent CD203c upregulation and CD63 induction even at lower concentration of Fel d1 (e.g., 0.002 $\mu\text{g}/\text{ml}$) (Fig. 2A, upper row) or E4.15 (e.g., 0.2 $\mu\text{g}/\text{ml}$) (Fig. 2A, middle row). Both CD203c and CD63 expression was progressive to higher levels with the increased Fel d1 (e.g., 0.02–0.2 $\mu\text{g}/\text{ml}$) or E4.15 concentration (e.g., 2–20 $\mu\text{g}/\text{ml}$) (Fig. 2A, 2E). However, a substantial population of the CD203c-positive basophils remained CD63 negative in all the Fel d1 concentration tested, indicating that not all the CD203c-positive cells would coexpress the anaphylactic degranulation marker CD63 upon optimal Fel d1 stimulation. In normal healthy donors ($n = 5$), PAE and E4.15 also promoted apparent basophil CD203c and CD63 expression in a similar fashion as that in cat-allergic donors (Fig. 2B, 2F, upper and middle rows). These results collectively demonstrated that only a portion of the CD203c-positive basophils undergoes anaphylactic degranulation induced by strong stimuli such as allergen, PAE, or high-affinity anti-IgE mAb. Therefore, CD203c expression as a basophil activation marker would always have higher sensitivity compared with that of CD63 expression (Fig. 2E, 2F), but not necessarily reflect the anaphylactic degranulation status.

LAIGE p6.2, however, weakly upregulated CD203c expression, compared with that promoted by Fel d1, PAE, or E4.15, from both cat-allergic and normal healthy donors, in a p6.2 dose-dependent fashion under the relatively high concentration (e.g., 2–20 $\mu\text{g}/\text{ml}$) (Fig. 2C, 2D), but the LAIGE-promoted CD203c-positive cells did not coexpress CD63 (Fig. 2A, 2B, lower rows). LAIGE-induced CD203c expression appeared to be rate limited as even higher LAIGE doses (up to 100 $\mu\text{g}/\text{ml}$) would not further increase CD203c expression to the level driven by low concentration of allergen or high-affinity anti-IgE mAbs (data not shown). Histamine release level of LAIGE p6.2–treated blood of both cat-allergic and normal healthy donors showed no difference from the spontaneous release level (Fig. 2G, 2H). As a positive control, allergen Fel d1, PAE, and E4.15 mAb triggered apparent histamine release (Fig. 2G, 2H). In addition, LAIGE p6.2 at concentration of 0.2, 2, and 20 $\mu\text{g}/\text{ml}$ did not stimulate IL-4 secretion for 1-, 2-, and 4-h incubation ($n = 3$) (Fig. 3B). These results demonstrated that allergen or high-affinity anti-IgE triggered basophil anaphylactic degranulation, whereas LAIGE p6.2 induced neither basophil anaphylactic degranulation nor IL-4 secretion, albeit a weak CD203c upregulation was promoted by higher concentration.

Determination of the relationship between anti-IgE mAb affinity and their capacity to induce basophil CD63 expression

To further test the hypothesis that anti-IgE Abs would display differential basophil activation capability based on their affinity to

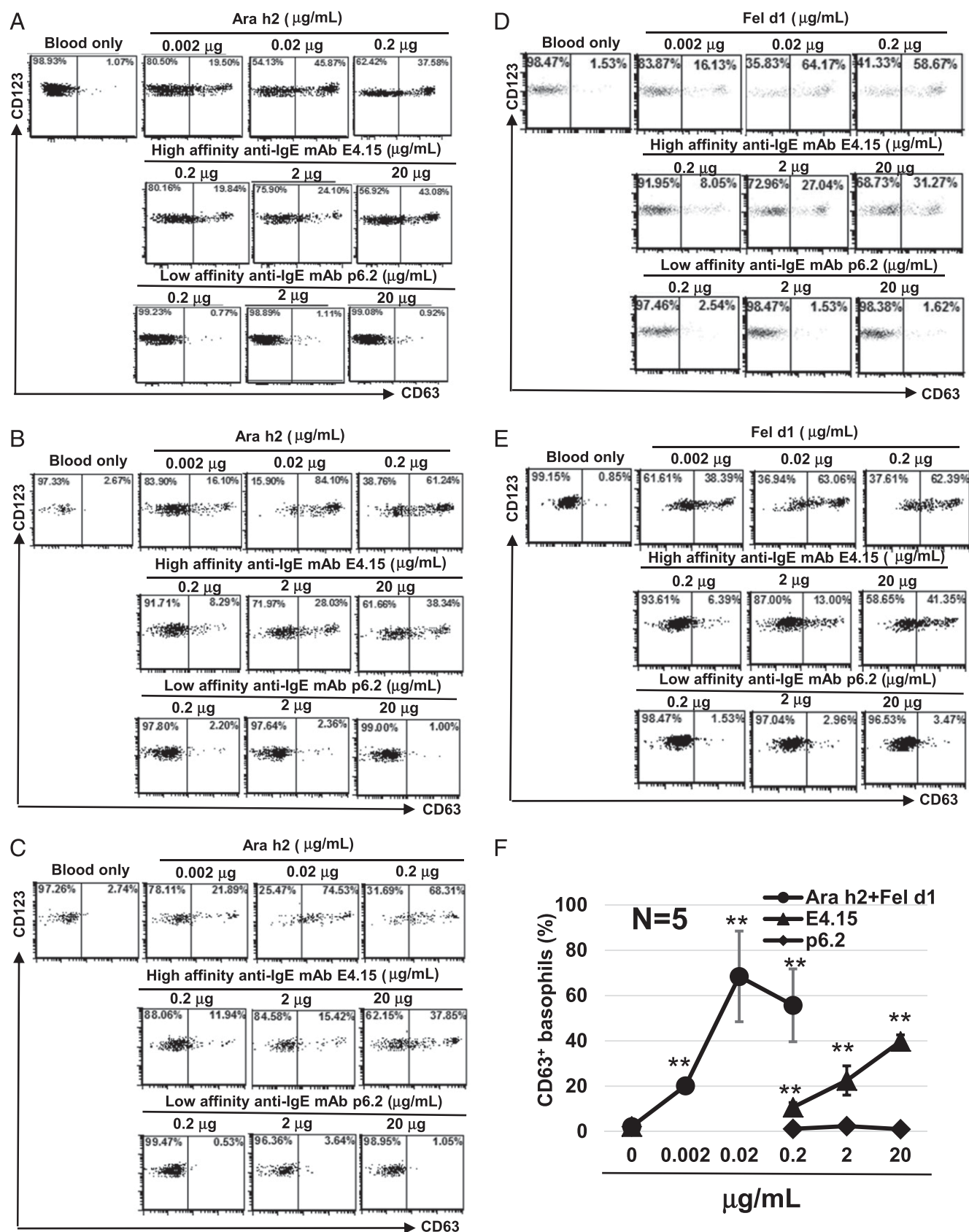


FIGURE 1. Basophil CD63 expression profiles of the allergic subjects induced by allergens and anti-IgE Abs. Three-color FACS analysis was employed, and the CD123⁺/HLA-DR⁻ population was gated as basophils for measurement of CD63 expression. (A–C) The dose-response basophil CD63 expression of three peanut-allergic subjects induced by Ara h2, E4.15, and LAIGE p6.2. (D and E) The dose-response basophil CD63 expression profiles of two cat-allergic subjects. (F) Summary of the kinetic dose-response curves of CD63 expression to allergens (Ara h2 + Fel d1), E4.15 and p6.2 ($n = 5$).

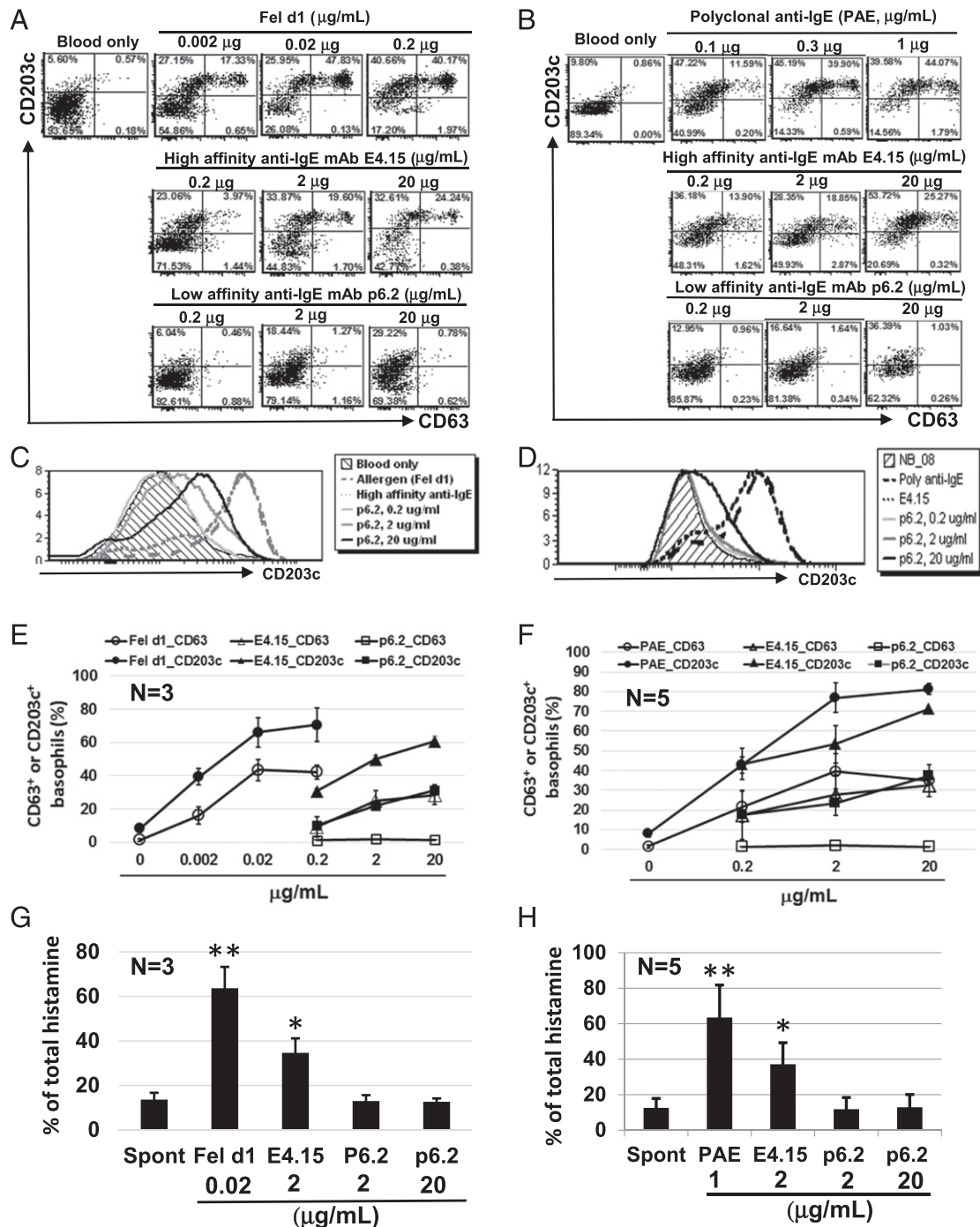


FIGURE 2. Basophil CD63 and CD203c expression profiles of the cat-allergic and normal healthy subjects induced by Fel d1 and anti-IgE Abs. Four-color FACS analysis was employed to simultaneously determine the basophil CD63 and CD203c expression. The CD123⁺/HLA-DR⁻ population was gated as basophils. **(A)** The dose-response basophil CD63/CD203c expression of the cat-allergic subjects ($n = 3$) induced by Fel d1, E4.15, and LAIGE p6.2. **(B)** The dose-response basophil CD63/CD203c expression of the normal healthy subjects ($n = 5$) induced by PAE, E4.15, and LAIGE p6.2. **(C and D)** Dose-dependent CD203c upregulation promoted by LAIGE p6.2 in both cat-allergic and normal healthy subjects, respectively (solid lines). The dotted lines represent the maximal CD203c upregulation driven by Fel d1, PAE, and E4.15 for comparison. **(E and F)** Summary of the kinetic dose-response curves of CD63 (open symbols) and CD203c (filled symbols) expression driven by Fel d1, E4.15, and LAIGE p6.2. **(G and H)** Histamine release levels from cat-allergic ($n = 3$) and normal subjects ($n = 5$).

IgE, we surveyed an array of the anti-human IgE mAbs for their ability to activate basophils, while measuring their corresponding binding affinity to IgE by surface plasmon resonance (26). The kinetic CD63 expression induced by six anti-IgE mAbs with different affinities to IgE is presented in Fig. 3A, whereby the relationship between IgE affinity and basophil activation can be directly assessed. The high-affinity anti-IgE mAbs E4.15, E7.12,

C6, and E5.1, with K_d in 10^{-9} to 10^{-10} M range, all induced robust CD63 expression at the concentration $<2 \mu\text{g/ml}$. In contrast, the LAIGE mAb p6.2 and E2.18, with K_d in 10^{-6} to 10^{-8} M range, did not induce CD63 expression at the concentrations $<40 \mu\text{g/ml}$, and only sometimes induced low-level CD63 expression at concentrations $>60 \mu\text{g/ml}$ with E2.18 (Fig. 3A, Supplemental Fig. 2A). This comparative study revealed that the

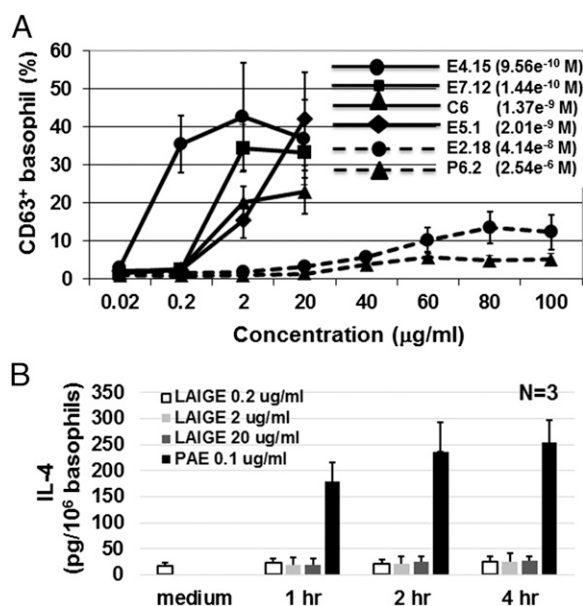


FIGURE 3. Basophil degranulation-triggering capacity of anti-IgE mAbs. **(A)** Dose-response BAT of anti-IgE mAbs with differential affinities to IgE ($n = 3$). **(B)** Basophil IL-4 production induced by LAIGE p6.2, with PAE as a positive control ($n = 3$).

anti-IgE Ab affinity was tightly correlated with their ability to trigger basophil anaphylactic degranulation, with high- but not low-affinity anti-IgE mAbs being capable of robustly triggering basophil activation and anaphylactic degranulation.

Determination of allergic-triggering capacity of LAIGE in hFcεRIα Tg mice

As both LAIGE p6.2 and E2.18 failed to trigger apparent basophil CD63 expression (Figs. 1–3, Supplemental Fig. 2A), to evaluate safety profile of LAIGE as a potential allergy therapeutic, we further examined whether LAIGE would trigger degranulation of allergic effector cells employing other available experimental assays, including IgE-mediated PCA reflecting mast cell degranulation, and systemic anaphylaxis reflecting both basophil and mast cell function combined, to rigorously evaluate the ability of LAIGE to trigger allergic reactions with higher dose, using p6.2 as a prototype of LAIGE.

For PCA, the hFcεRIα Tg mice were systemically sensitized with human myeloma IgE (PSIgE), followed by local challenge with increasing amounts of p6.2 (Fig. 4A). As a positive control, PAE at 1 μg/ml induced a strong skin reaction (low left corner, Fig. 4A). However, p6.2 did not trigger PCA reactivity at doses up to 60 μg/ml, and weak reactivity was seen at doses of 80–100 μg/ml (Fig. 4A, 4B).

To test whether p6.2 induced in vivo systemic anaphylaxis, hFcεRIα Tg mice systemically sensitized with human PSiGE (50 μg per mouse) were challenged with 50 and 250 μg p6.2, or 50 μg of E4.15 (equivalent 2 μg/g body weight) as a positive control. The expected fall in core body temperature was seen in the mice challenged with E4.15, but not in those challenged with 50 (data not shown) and 250 μg p6.2 (Fig. 4D). Correspondingly, a positive anaphylactic clinical score (38) was only observed in E4.15- but not in p6.2-challenged mice (Fig. 4C). In a separate experiment to measure allergic mediator release with multiplex luminex assay in serum samples following p6.2 challenge, mouse mast cell protease-1 release level was minimal compared with the positive control challenged with E4.15 (Fig. 4E). The serum IL-4, IL-5, IL-10, and IL-13 levels were also not substantially different

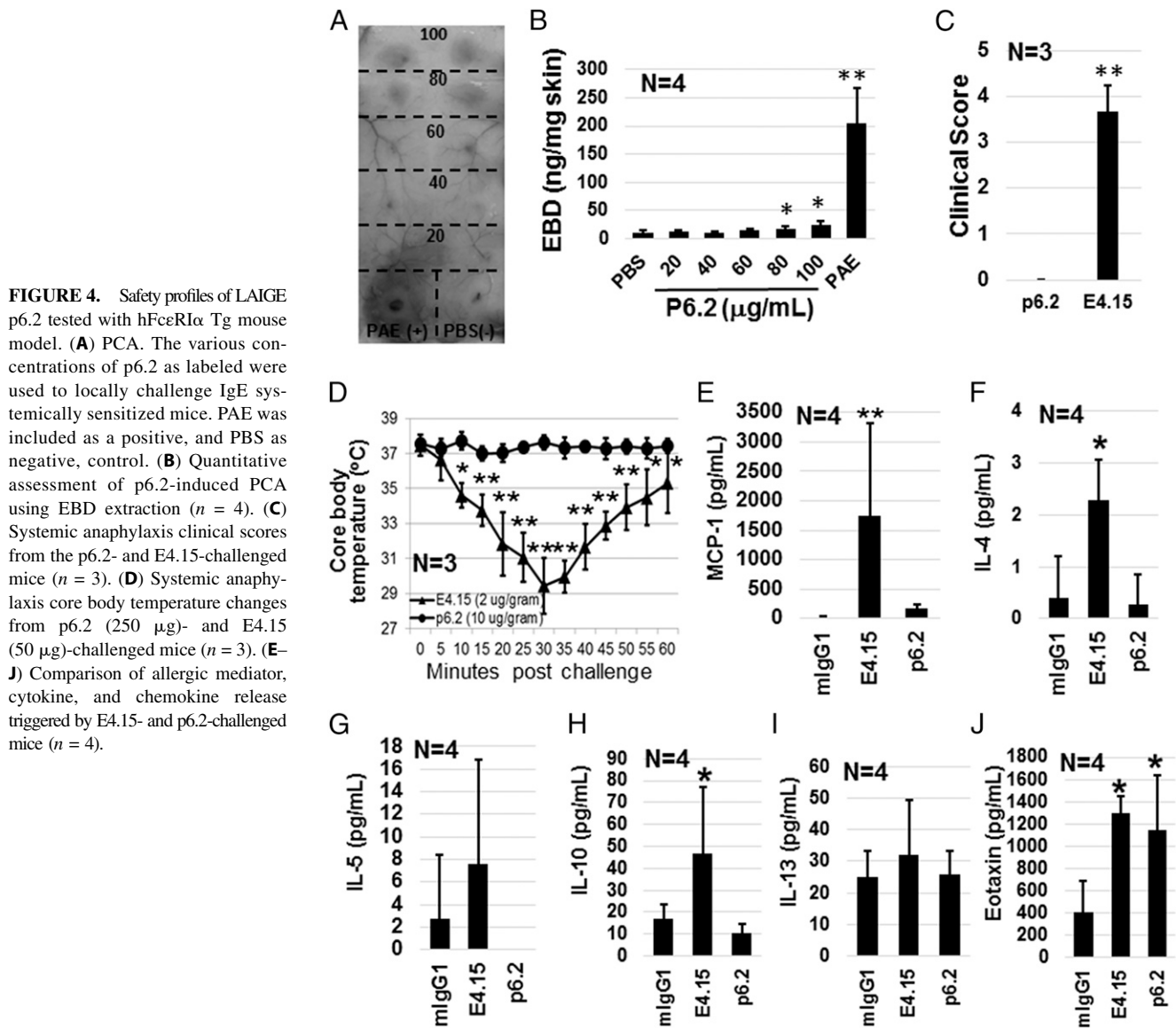
between p6.2- and mIgG1-challenged mice (Fig. 4F–I). However, p6.2 appeared to induce eotaxin secretion to the level comparable to that of E4.15 challenge (Fig. 4J). Collectively, these data indicated that p6.2 did not show anaphylactic activity at doses <60 μg/ml, and might display weak degranulation activity with the doses >60 μg/ml, and might selectively affect certain chemokine secretion at high concentration.

LAIGE blocks peanut and cat allergen-induced basophil anaphylactic degranulation

A modified BAT assay capable of detecting basophil CD63 induction with prolonged incubation time (24 or 48 h) was used to test the effectiveness of LAIGE to block allergen-induced basophil anaphylactic degranulation. Although compromised, the basophil CD63 induction of allergic blood by either peanut or cat allergens was largely preserved for up to 48 h if the blood was incubated in ambient temperature (22–24°C) with slow agitation at 100 rpm. Such a modified BAT assay enables us to evaluate the therapeutic effects of LAIGE (or other agents with anti-allergy potential) ex vivo in a BAT-based assay format because of the 24- to 48-h incubation window. As peanut allergen- or Fel d1-induced basophil CD63 expression did not apparently affect by incubation with p6.2 for up to 8 h (data not shown), the whole blood from peanut- or cat-allergic subjects was incubated for 24 or 48 h with various amounts of p6.2 or E2.18, with mIgG1 as an isotype control, followed by challenging with the corresponding allergens for basophil CD63 induction. Because 48-h incubation of blood would frequently have CD63^{low} background stain (Fig. 5), we only focused on assessing the CD63^{high} expression (gated in circle in Fig. 5) in this modified BAT. Peanut allergen (Ara h1, h2, and h6 combined)-induced basophil CD63^{high} expression was inhibited in a dose-dependent manner by incubation with p6.2 for 48 h, but not mIgG1, in 0.5–4 μg/ml range (Fig. 5A), with ~90% inhibition achieved at 2–4 μg/ml in all five peanut-allergic blood tested (Fig. 5B). Similarly, Fel d1-induced basophil CD63^{high} expression from cat-allergic donors' blood was also significantly blocked in a similar scale to that in peanut-allergic donors, also in a dose-dependent fashion (Fig. 5C, 5D). Fel d1-induced CD63^{high} expression was also blocked by E2.18 in a dose-dependent manner (Supplemental Fig. 2B). These data demonstrate that LAIGE p6.2 and E2.18 at relatively lower doses could effectively block both peanut- and cat-allergic IgE-mediated CD63 expression indicative of anaphylactic degranulation.

LAIGE exhibits a higher safety profile than that of high-affinity anti-IgE mAbs

To quantitatively assess the therapeutic potential and safety profile of LAIGE mAbs in IgE-mediated allergies, we used a BAT-based therapeutic index to evaluate the safety of the LAIGE-based allergy therapy, with the high-affinity anti-IgE mAbs as a comparison. The therapeutic index of the peanut BAT-based approach was calculated as the BAT triggering dose divided by the BAT effective therapeutic dose (Fig. 6). The BAT triggering dose was arbitrarily defined as the lowest anti-IgE mAb concentration capable of inducing >5% basophil CD63 expression, whereas the effective therapeutic dose was arbitrarily defined as the lowest anti-IgE mAb dose sufficient to block >50% of peanut allergen-triggered CD63 expression. Thus, the higher the therapeutic index, the safer the therapy. As expected, the LAIGE mAb E2.18 and p6.2 exhibited a much larger therapeutic index compared with the high-affinity anti-IgE mAb E4.15, E7.12, C5, and E5.1, which displayed much smaller therapeutic index (Fig. 6), indicating that the LAIGE mAbs as an allergy therapeutic are much safer compared with that of the high-affinity anti-IgE mAbs.



LAIGE blocks IgE-mediated PCA

We used the previously established PCA assay (16) to test whether LAIGE p6.2 was able to block mast cell-mediated allergic reactivity. Although PCA was a short-term assay, we found that the passively skin-sensitized IgE would last for up to 4 d for reliable PCA reaction (data not shown). The hFcεRIα Tg mice were skin sensitized with serially diluted peanut-, cat-, and/or dansyl-specific IgE (from 0.125 to 2 μ g/ml) for 2 h, treated i.p. for 2 and 4 d, respectively, with LAIGE p6.2 or control mIgG1, followed by systemic i.v. challenge with corresponding allergens at day 4 for assessing PCA reaction by quantifying the extravasated EBD in PCA spots from the LAIGE-treated and controlled mice, respectively. p6.2, at 2 μ g/g body weight, did not apparently affect PCA reaction after p6.2 treatment for 2 d (data not shown), but significantly blocked peanut-allergic IgE (Fig. 7A, 7B)-, cat-allergic IgE (Fig. 7C, 7D)-, and dansyl-specific IgE (18) (Fig. 7E, 7F)-mediated PCA reactivity after 4-d treatment. Cat-allergic IgE-mediated PCA was also strongly blocked by E2.18 after 4-d treatment (Supplemental Fig. 2C). Collectively, these data demonstrated that p6.2 exhibited a slower onset (>2 d) for skin mast cell desensitization, and the desensitized status was maintained at least up to 4 d after LAIGE was administered.

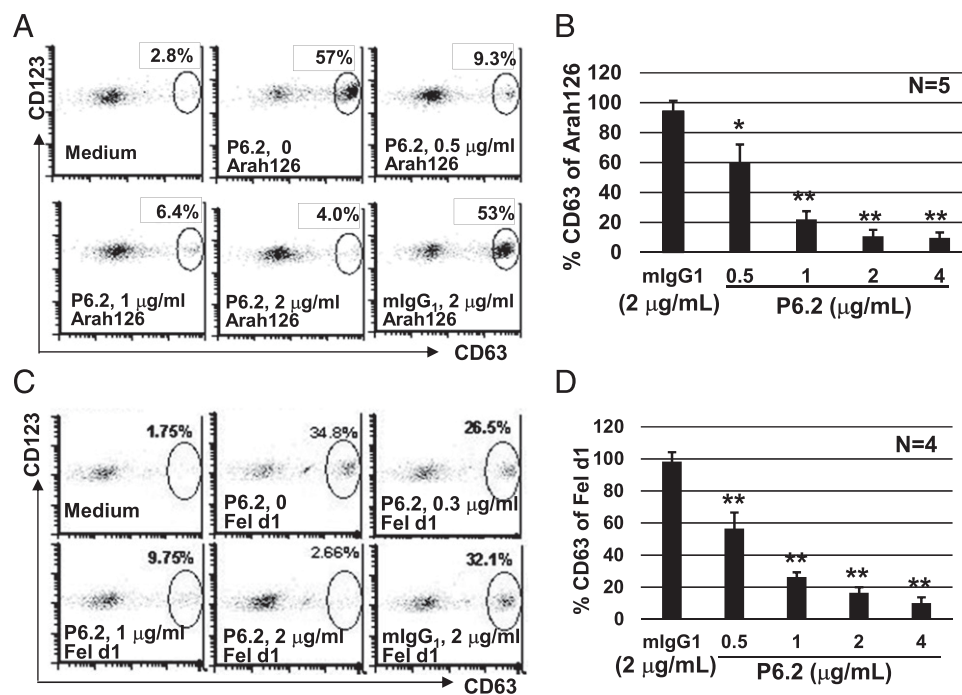
LAIGE p6.2 attenuates dansyl IgE-mediated systemic anaphylaxis

To test the effect of LAIGE on systemic anaphylaxis, the hFcεRIα Tg mice were systemically sensitized with the recombinant dansyl-specific human IgE, followed by treatment with p6.2 or a mIgG1 isotype control. Four days later, the mice were i.v. challenged with the modal allergen dansyl-BSA, and the animals' core body temperature changes and systemic anaphylaxis clinical index were recorded as measures of systemic anaphylaxis (20, 21). Dansyl-BSA induced a significant drop in core body temperature (Fig. 8A), and a high anaphylactic clinical score (Fig. 8B), in the mIgG1 isotype control mice. In contrast, the markedly reduced clinical scores and temperature changes reflecting systemic anaphylaxis were observed in the p6.2 (2 μ g/g body weight)-treated group (Fig. 8), clearly indicating that p6.2 attenuated dansyl-specific IgE-mediated systemic anaphylaxis.

p6.2 downregulates basophil and mast cell surface IgE and FcεRI expression

To gain insight into the mechanism by which LAIGE mAbs suppress allergic reactivity, we examined whether the LAIGE p6.2 altered surface IgE and FcεRI expression on allergic effector cells.

FIGURE 5. LAIGE p6.2 blocks allergen-induced basophil CD63 expression. **(A)** FACS profile of the peanut allergen-induced CD63^{high} expression inhibited by p6.2. **(B)** Summary of the inhibitory effects of p6.2 on peanut allergen-induced CD63^{high} expression ($n = 5$). **(C)** FACS profile of the cat allergen Fel d1-induced CD63^{high} expression inhibited by p6.2. **(D)** Summary of the inhibitory effects of p6.2 on cat allergen Fel d1-induced CD63^{high} expression ($n = 4$).



To this end, basophils (CD123⁺/HLA-DR⁺) from blood PBMCs (which were in vivo sensitized with IgE) were treated with p6.2 or mIgG1 isotype control, and the surface IgE/Fc ϵ RI levels were determined. Fig. 9 is a representative experiment showing that the IgE and Fc ϵ RI levels, but not that of CD123 (IL-3R α), were decreased by treatment of p6.2 in a dose-dependent manner in the dose range of 1–5 μ g/ml (Fig. 9A).

To define the effects of p6.2 on mast cell surface IgE and Fc ϵ RI expression, the hFc ϵ RI α Tg mouse BMMCs were sensitized with human myeloma IgE and incubated with p6.2 or the mIgG1 isotype control for 4 d, and then the surface level of IgE and Fc ϵ RI was assessed by staining with FITC-labeled anti-human IgE mAb E4.15 (which does not compete with p6.2 for IgE binding) and PE anti-human Fc ϵ RI α . The c-kit (CD117) served as an internal staining control. Fig. 9B shows that both surface IgE and Fc ϵ RI were markedly decreased by p6.2 in a dose-dependent manner. These data documented that both basophil and mast cell surface IgE and Fc ϵ RI were downregulated by p6.2 treatment.

p6.2 directly mediates internalization of surface IgE

Because surface IgE and Fc ϵ RI were downregulated by p6.2, we used confocal microscopy to define whether p6.2 drove internalization of surface IgE. FITC-IgE-sensitized hFc ϵ RI α ⁺ BMMCs were treated with the following: 1) mIgG1 isotype control; 2) 2 μ g/ml p6.2 for 24 and 48 h; and 3) PAE as a positive control. Following mIgG1 treatment, IgE remained stably bound on the surface at 24 (data not shown) and 48 h, being detected as bright rings on the periphery of BMMCs with minimal internalization (minimal green signals inside the cells; left panel of Fig. 10A). The bright surface rings became markedly dimmer in BMMCs treated with p6.2 for 48 h; instead, numerous fine green signals appeared inside the cells (middle panel, Fig. 10A). Confocal sections from top to bottom of the p6.2-treated BMMCs demonstrated that the fine green signals were located intracellularly, being distributed throughout all eight sections selected (Fig. 10B, enlarged from the highlighted area of the middle panel of Fig. 10A). The p6.2-induced internalization of surface IgE exhibited a strikingly different pattern compared with that triggered by PAE. In the latter case, large aggregates and a capping

and polarized distribution of surface IgE were seen (right panel, Fig. 10A). These data revealed that surface IgE has undergone internalization triggered by p6.2 treatment.

p6.2-triggered IgE internalization is colocalized with Fc ϵ RI

Confocal microscopic analysis was employed to define whether surface IgE was internalized with Fc ϵ RI as the mechanism responsible for p6.2-driven Fc ϵ RI downregulation. The hFc ϵ RI α ⁺ BMMCs were first sensitized with FITC-IgE (showed green), treated for 24 or 48 h with 2 μ g/ml p6.2 or an mIgG1 isotype control, followed by intracellular staining with Alexa fluoro-647-labeled anti-human Fc ϵ RI α (showed red). The mIgG1-treated BMMCs displayed bright surface as well as confined intracellular compartment Fc ϵ RI staining, including the areas surrounding the nucleus (Fig. 10C1, 10C7), whereas IgE was mainly on the surface with little appearing intracellularly (Fig. 10C2, 10C8).

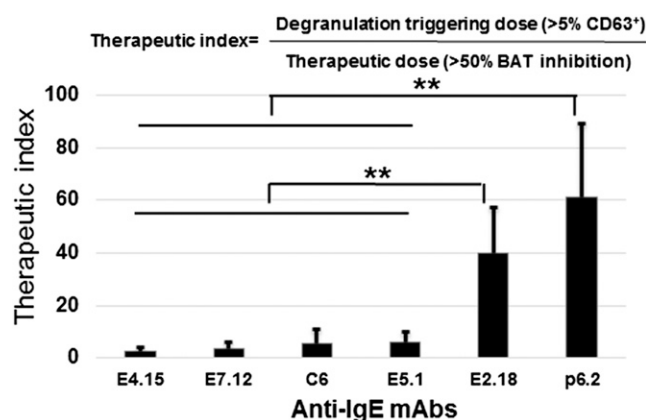
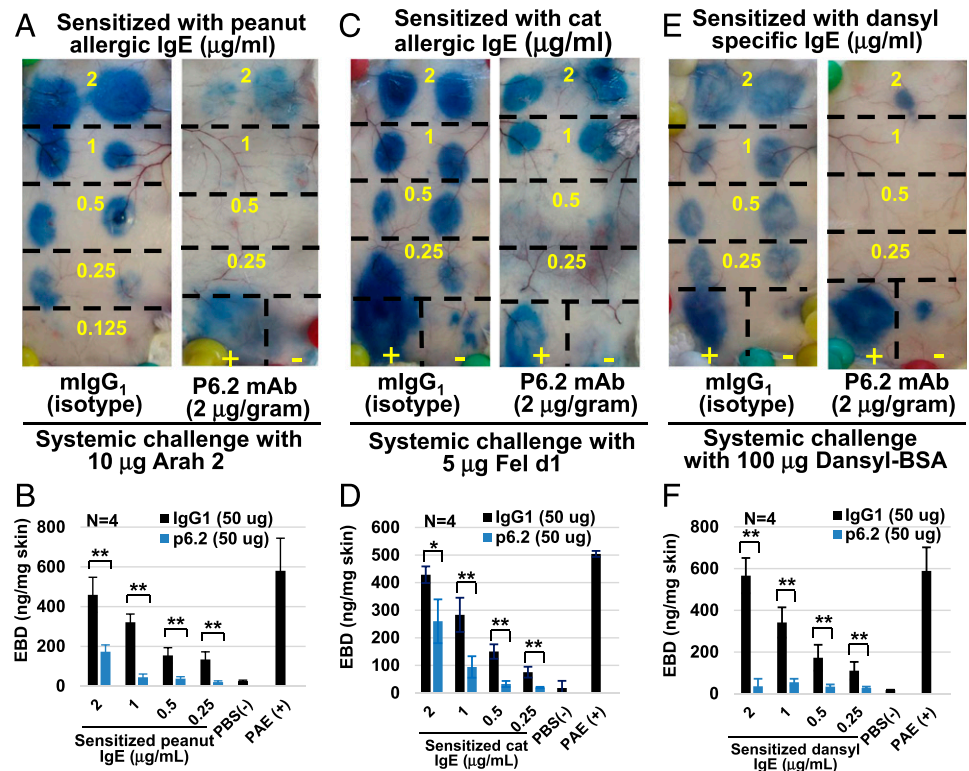


FIGURE 6. The therapeutic index of high- and low-affinity anti-IgE mAbs. The therapeutic index of the peanut BAT-based approach was calculated as degranulation triggering dose divided by the therapeutic dose. The BAT triggering dose was defined as the lowest anti-IgE mAb concentration capable of inducing >5% basophil CD63 expression, whereas the effective therapeutic dose was arbitrarily defined as the lowest anti-IgE mAb dose sufficient to inhibit >50% of peanut allergen-triggered CD63 expression ($n = 3$).

FIGURE 7. LAIGE p6.2 blocks IgE-mediated PCA in hFcεRIα Tg mice. **(A)** p6.2 inhibited peanut-allergic IgE-mediated PCA. **(B)** Quantitative EBD assessment from the peanut-allergic IgE-mediated PCA spots. **(C and D)** p6.2 inhibited cat-allergic IgE-mediated PCA. **(E and F)** p6.2 inhibited dansyl-specific IgE-mediated PCA. The plus (+) was the PAE positive control, whereas the minus (−) was the PBS negative control. $n = 4$ mice for each group.



FcεRI was primarily colocalized with IgE on the surface areas (shown as yellow on merge in Fig. 10C3, 10C9), without apparent intracellular colocalized signals. In contrast, a weak surface IgE signal was seen on the p6.2-treated BMMCs, accompanied by a significantly increased intracellular IgE signal (Fig. 10C2, 10C5, 10C8, 10C11), indicating that the surface IgE had undergone internalization. The intracellular IgE in the p6.2-treated BMMCs was mainly colocalized with FcεRI (Fig. 10C6, 10C12), suggesting that p6.2 drove internalization of IgE–FcεRI complexes. Taken together, these data provide evidence for a mechanism by which p6.2 mediates downregulation of both surface IgE and FcεRI on BMMCs.

Discussion

Although direct targeting of the FcεRI-bound IgE on basophils/mast cells with an anti-IgE Ab as an allergy therapeutic had generally not been considered feasible as anti-IgE Abs were expected to cross-link surface IgE/FcεRI complexes and lead to allergic reactivity, Finkelman and colleagues (8) discovered that some anti-IgE and anti-FcεRI Abs could briefly induce allergic effector cell desensitization, but not allergic degranulation. In addition, naturally occurring anti-IgE autoantibodies were found to inhibit allergen-induced basophil activation (9), and the anti-IgE and anti-FcεRIα autoantibodies in chronic urticaria patients have been found to render basophils unresponsive to following anti-IgE stimulation (10). These studies support the concept that not all anti-IgE Abs are created equal in terms of their capacity of triggering allergic degranulation; instead, some anti-IgE Abs would play a role in allergic desensitization, albeit the mechanism(s) by which these anti-IgE Abs render allergic desensitization was not known currently. Thus, anti-IgE Abs with the property of desensitizing allergic effector cells would have the potential as a novel allergic therapeutic.

In this study, we find that LAIGE p6.2 and E2.18 are able to profoundly block allergic responses without triggering anaphylactic degranulation in both static and dynamic models of allergic

reactivity. In contrast to allergens and/or the high-affinity anti-IgE Abs that cross-link the surface IgE/FcεRI and induce anaphylactic degranulation, LAIGE mAbs fail to trigger anaphylactic degranulation and cytokine production of allergic effector cells even at high concentrations. This is presumably due to its weak interaction with and/or fast dissociation from the FcεRI-bound IgE such that the stable FcεRI cross-linking required for transduction of the anaphylactic degranulation is not formed during the interaction of LAIGE with the surface-bound IgE. This feature of LAIGE mAb interaction with surface-bound IgE provides the basis for its safety

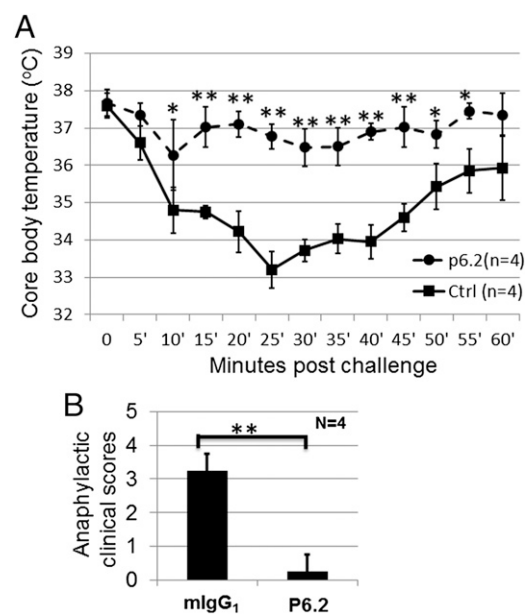
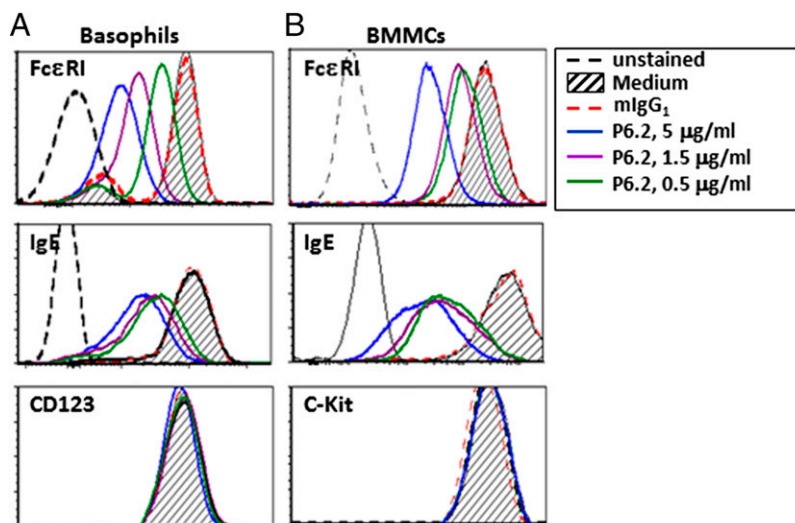


FIGURE 8. p6.2 attenuates dansyl IgE-mediated systemic anaphylaxis. **(A)** The rectal temperature changes of the dansyl IgE-sensitized hFcεRIα Tg mice in control (mIgG1) and p6.2-treated mice ($n = 4$). **(B)** The anaphylactic clinical scores in above two groups of mice.

FIGURE 9. Downregulation of surface IgE and FcεRI on basophils and BMMCs by p6.2. **(A)** Effects of p6.2 on expression of the basophil surface FcεRI and IgE levels determined by flow cytometry. CD123 staining was included as an internal staining control. The data shown are the representative of three experiments. **(B)** Effects of p6.2 on the BMMC surface FcεRI and IgE expression. The c-kit staining was included as an internal staining control. Shown are the representative data from two experiments.



as a novel approach to suppress allergic reactions in contrast with the high-affinity anti-IgE Ab that will generally trigger allergic anaphylactic degranulation immediately even in low dose. Instead, the weak and/or transient binding of p6.2 to the FcεRI-bound IgE promotes internalization of the surface IgE-FcεRI complex, leading to rapid downregulation of the surface IgE/FcεRI that correlated with LAIGE's allergic blockade effects. Therefore, we speculate that LAIGE-mediated downregulation of the surface IgE-FcεRI might be one of the mechanisms responsible for LAIGE's allergic desensitization activity.

In contrast to the classic desensitization achieved through extremely low dose of allergens, such as with the traditional allergen

immunotherapy, and/or anti-IgE Abs that induced rapid but short-lived desensitization within a matter of minutes (8, 27, 28), LAIGE-induced allergic desensitization displayed quite distinct patterns and kinetics. First, onset of the LAIGE-induced desensitization appeared taking much longer time, for example, >8 h for human BAT, and >2 d for skin mast cell PCA and systemic anaphylaxis in hFcεRIα Tg mouse model. Second, the LAIGE-induced desensitization status appeared long lasting, for at least >24 h for basophils (as demonstrated in BAT), and >2 d for skin mast cells (as demonstrated in PCA and systemic anaphylaxis); thus, LAIGE-induced desensitization status was directly translated into the allergic blockade effect in all the experimental models

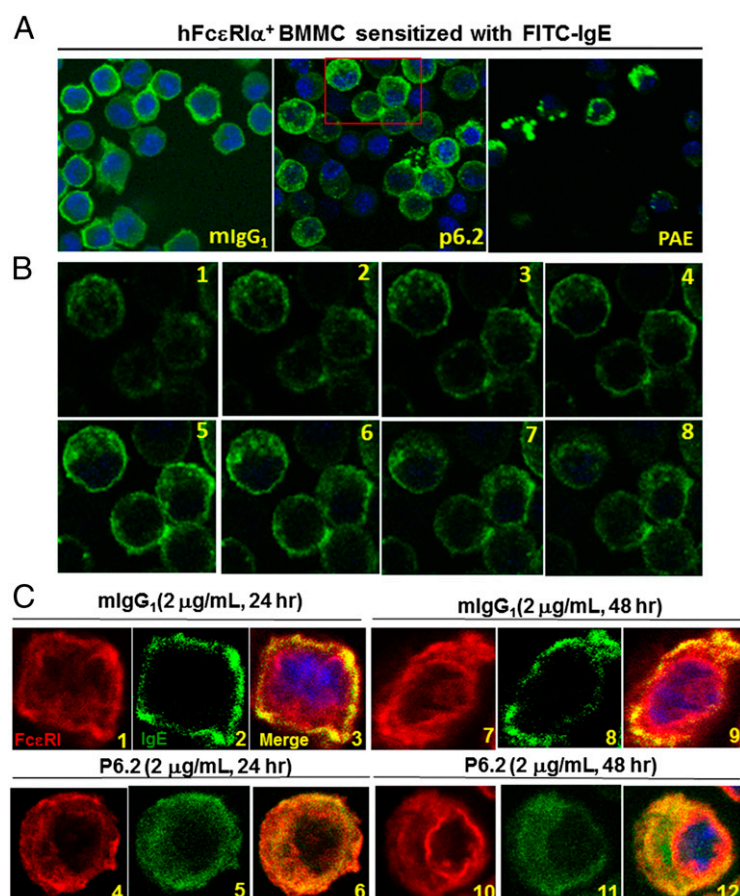


FIGURE 10. p6.2 triggers the surface IgE internalization and colocalization with FcεRI. **(A)** Effect of p6.2 on promoting surface IgE internalization. **(B1–B8)** Internalized FITC-IgE in the confocal sections. The highlighted area of the middle panel of (A) was subjected to confocal section analysis. Data are representative of two experiments with similar results. **(C)** Colocalization of IgE with FcεRI in the cytoplasmic compartment of BMMCs. The FcεRI was stained as red, IgE as green, and the colocalization of red and green merges as yellow, whereas the nucleus was stained as blue. The BMMCs were treated for 24 (C1–C6) and 48 (C7–C12) h, respectively. The data are the representative of >20 cells from each condition analyzed.

tested. As the limit of the relatively short-term nature of human BAT, hFcεRIα Tg mouse-based PCA, and passive systemic anaphylaxis used in this study, the even longer-term therapeutic effects could not be tested with these experimental models. Third, whereas allergen(s) and/or high-affinity anti-IgE Abs induced both basophil CD203c and CD63, LAIGE only selectively promoted weak CD203c but not CD63 expression even with high concentration. The latter was particularly interesting because basophil CD63 expression was directly associated with anaphylactic degranulation pathway, whereas CD203c expression had been implicated in the piecemeal degranulation pathway (13, 22, 23). Given that LAIGE selectively promoted CD203c expression, and CD203c expression was not associated with preformed mediator (e.g., histamine) release and cytokine IL-4 production from basophil (23) (Figs. 2G, 2H, 3B), it is tempting to hypothesize that selective activation of piecemeal degranulation pathway might be another mechanism involved in LAIGE-mediated allergic desensitization. Although the piecemeal degranulation pathway in basophil/mast cell is less understood, it was demonstrated that such a piecemeal degranulation pathway is most likely a general mediator secretory pathway regulating physiological function of eosinophils (29).

It is also anticipated that the weak association of the LAIGE mAb with soluble IgE in the extracellular compartment *in vivo* will allow the LAIGE mAb released from the anti-IgE/IgE complex to function as an active drug. Thus, the binding of LAIGE mAb to serum IgE, rather than neutralizing its therapeutic effects, is expected to provide a reservoir of active drug based on the reversible equilibrium of the low-affinity interaction with serum IgE. Such a dynamic process is predicted to extend the pharmacologic effects of the LAIGE mAbs and allow longer dosing intervals. Additionally, it should provide another layer of safety by buffering the anti-IgE with soluble IgE, so that upon administration, immediate direct contact of the administered anti-IgE with surface-bound IgE would not occur. Therefore, the level of the pre-existing IgE in serum would not likely be a limiting effect for p6.2's therapeutic effect as it is for omalizumab; instead, serum IgE will actually enhance/extend the action of the LAIGE-based therapy.

Historically, low-affinity protein–protein binding, or weak protein–protein interaction, was considered physiologically irrelevant. However, emerging evidence suggests that weak protein–protein interaction, which is not necessarily of low specificity (in fact, low-affinity interaction often exhibits high specificity), is fundamentally important for promoting the rapid on/off switching of signal transduction, reversible cell–cell contacts, transient assembly/disassembly of signaling complexes, and dynamic regulation of enzymes' activity for broad substrate specificity (11, 24, 30, 31). For example, the interactive affinity between lymphocytes' surface molecules is known to be very low with K_d in the 2×10^{-4} to 10^{-6} M range. Such low affinity is favorable for reversible cell–cell adhesion processes and yet sufficient to transduce biologically important signaling with high specificity (25). By comparing the high- and low-affinity stimuli to elicit cellular responses of mast cells via the FcεRI receptor, it was found that although similar receptor phosphorylation was achieved, the receptor cluster size, mobility, distribution, and the effector responses, including mediator release, were markedly different between the high- and low-affinity stimuli through preferentially activating separate signal pathways (32).

In the field of Ab-based biologics, specifically designing low-affinity mAbs as a therapeutic approach has not been previously considered. Our studies demonstrating that LAIGE mAbs have potent anti-allergic efficacy in inhibiting allergic effector cells underscore the concept that low-affinity Ab-based approaches may

have important overlooked therapeutic potential for allergy. As IgE/FcεRI provides the initial trigger for the allergic signaling cascade, it is not surprising that the effects of downregulation of the surface IgE and FcεRI would translate into major attenuation of the downstream signaling cascade and ultimately the allergic effector response. Based on this feature, we anticipate that the LAIGE therapy would have broader application in treating and preventing allergic diseases.

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Disclosures

K.Z. and A.S. are both employees of Sixel Inc., which has licensed the intellectual property rights from University of California, Los Angeles. K.Z. and A.S. also have patent interest in the technology via their positions at University of California, Los Angeles.

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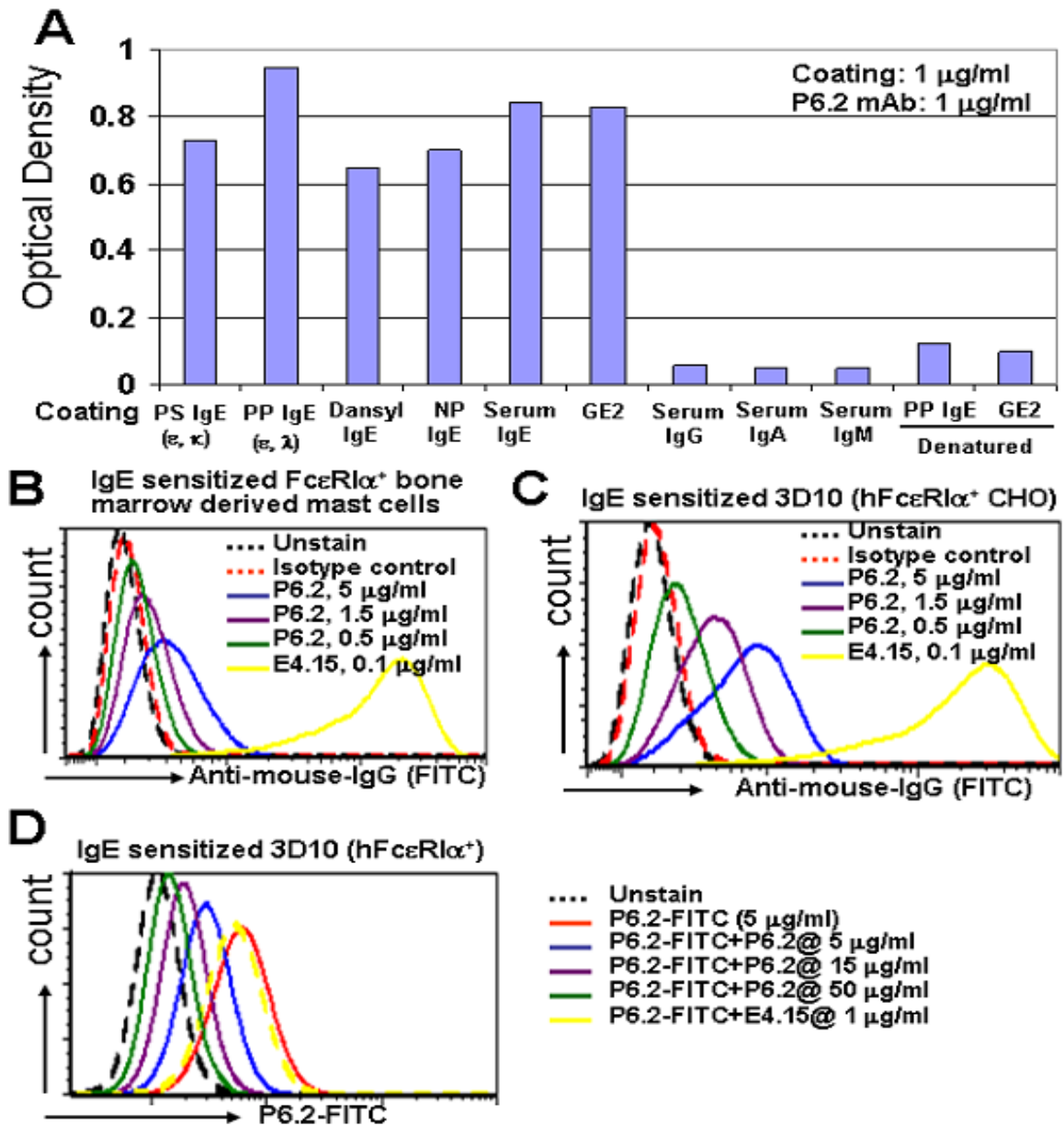


Figure S1. Characters of the LAAIGE p6.2 mAb. (A). IgE binding of p6.2 on ELISA format. p6.2 mAb (IgG₁, κ) binds to various human IgEs but not to human IgG, IgA, or IgM. p6.2's IgE binding activity for IgE is lost when IgE is denatured. (B). Surface IgE binding of p6.2 mAb on BMMC. p6.2 binds to the FcεRI-bound surface IgE on BMMCs in a dose dependent fashion. (C). Surface IgE binding of p6.2 mAb on human FcεRIα stably transfected CHO cell line 3D10 in a dose dependent fashion. (D). Binding of FITC-labeled p6.2 to the surface-bound IgE was specifically competed by unlabeled p6.2 in a dose-dependent fashion, but not by the anti-human IgE mAb E4.15.

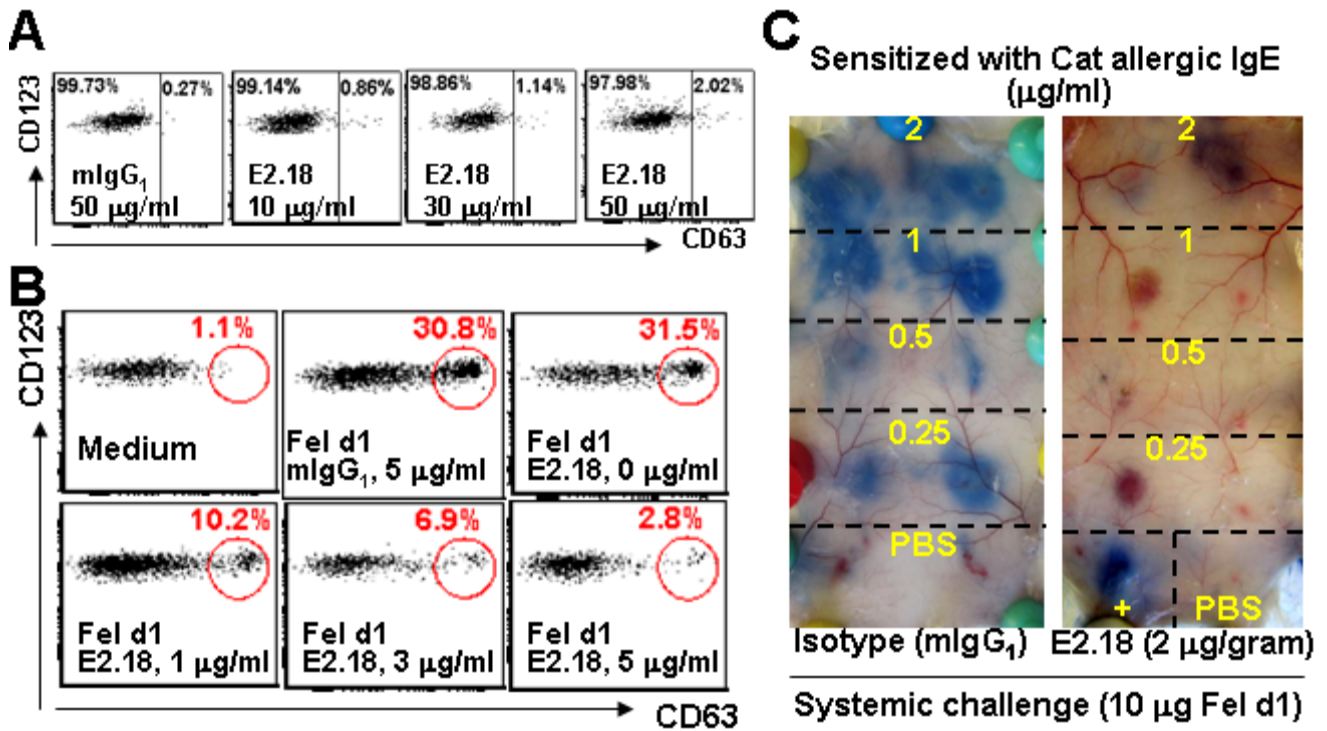


Figure S2. The safety profile and therapeutic effect of the LAAIGE mAb E2.18. (A). Effect of E2.18 mAb on basophil activation. A cat allergy subject's blood was incubated with various concentration of E2.18 for 15 minutes for basophil activation using standard BAT protocol. E2.18 mAb did not trigger apparent basophil activation up to the dose of 50 µg/mL. (B). Effect of E2.18 on Fel d1-induced cat BAT. The cat allergy blood was incubated with E2.18 at 0-5 µg/mL for 48 hours, followed by activation with Fel d1 at 0.02 µg/mL for 15 minutes. (C). Effect of E2.18 mAb on Fel d1-induced PCA. The FcεRIα Tg mice were locally sensitized with IgE purified from the cat allergic subject's plasma for 2 hours, and then injected with either control mIgG₁, or E2.18, at 2 µg/gram body weight for four days before subjecting to Fel d1 challenging with the standard PCA protocol.