Optimizing drug inhibition of IgE-mediated anaphylaxis in mice

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Background: Administering allergens in increasing doses can temporarily suppress IgE-mediated allergy and anaphylaxis by desensitizing mast cells and basophils; however, allergen administration during desensitization therapy can itself induce allergic responses. Several small molecule drugs and nutraceuticals have been used clinically and experimentally to suppress these allergic responses.

Objectives: This study sought to optimize drug inhibition of IgE-mediated anaphylaxis.

Methods: Several agents were tested individually and in combination for ability to suppress IgE-mediated anaphylaxis in conventional mice, FcεRIα-humanized mice, and reconstituted immunodeficient mice that have human mast cells and basophils. Hypothermia was the readout for anaphylaxis; therapeutic efficacy was measured by degree of inhibition of hypothermia. Serum mouse mast cell protease 1 level was used to measure extent of mast cell degranulation.

Results: Histamine receptor 1 (HR1) antagonists, β-adrenergic agonists, and a spleen tyrosine kinase (Syk) inhibitor were best at individually inhibiting IgE-mediated anaphylaxis. A Bruton's tyrosine kinase (BTK) inhibitor, administered alone, only inhibited hypothermia when FcεRI signaling was suboptimal. Combinations of these agents could completely or nearly completely inhibit IgE-mediated hypothermia in these models. Both Syk and BTK inhibition decreased mast cell degranulation, but only Syk inhibition also blocked desensitization. Many other agents that are used clinically and experimentally had little or no beneficial effect.

Conclusions: Combinations of an HR1 antagonist, a β-adrenergic agonist, and a Syk or a BTK inhibitor protect best against IgE-mediated anaphylaxis, while an HR1 antagonist plus a β-adrenergic agonist ± a BTK antagonist is optimal for inhibiting IgE-mediated anaphylaxis without suppressing desensitization. (J Allergy Clin Immunol 2021;■■■:■■■-■■■.)

Key words: *Desensitization, antihistamine, Syk, BTK, β-adrenergic agonist, mast cell, mouse, humanized mouse, FCεRI*

The considerable and increasing prevalence of allergic disorders[1-5] has served as an impetus for the development of novel therapies. Several of these therapeutic approaches involve desensitization, the concept that exposure to allergen doses that are insufficient to cause symptoms temporarily increases the allergen dose required to elicit symptoms.[6-12] For IgE-dependent allergy, desensitization is predominantly allergen-specific[8,13-16] and results from reversible allergen-induced changes in IgE/FcεRI expression,[17,18] actin conformation,[19] and signaling[15,18,19] on mast cells and basophils. Unlike true tolerance, allergen sensitivity recurs unless allergen exposure is repeated at frequent intervals.[7,9,11,19-22] Despite this limitation, desensitization with escalating doses of allergen, administered intravenously, orally, sublingually, or transcutaneously, is currently being used effectively to treat drug and food allergy.[7,23-29]

Allergen desensitization, however, whether the relatively slow approach that is most frequently used to treat food allergy[7] or the rapid approach used to treat drug allergy,[25] is not without risk. Patients undergoing this therapy, like patients being treated with more prolonged courses of subcutaneous allergen that can induce more persistent tolerance by inducing blocking IgG antibodies and promoting regulatory T- and B-cell responses, can develop local or systemic adverse responses when the therapy induces excessive mast cell or basophil activation.[7,12,23,27,29-35] To minimize these adverse responses during rapid desensitization for food allergy, it is common to treat patients prophylactically with corticosteroids, antihistamines that suppress histamine receptor 1 (HR1) and HR2, and leukotriene or leukotriene receptor antagonists.[36-42] With some exceptions,[42] however, the choice of these drugs is based more on theoretical considerations than on experimental results that demonstrate efficacy.

To optimize such prophylactic drug therapy during desensitization, we have used conventional and humanized mouse models to test the ability of different agents to protect against IgE-mediated anaphylaxis. Our results demonstrate additive or synergistic protective effects of HR1, but not HR2 antagonists, β-adrenergic agonists (but not epinephrine), a Bruton's tyrosine kinase (BTK) inhibitor, and a spleen tyrosine kinase (Syk) inhibitor (although the Syk inhibitor suppresses desensitization as well as anaphylaxis); in contrast, several other agents that have been described to protect against anaphylaxis had little or no protective effect against the development of hypothermia in our experiments.

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Abbreviations used
15.1: Mouse IgG_1 anti-human FcεRIα mAb–secreting hybridoma cells
BTK: Bruton's tyrosine kinase
DMSO: Dimethyl sulfoxide
EM-95: Rat IgG_{2a} anti-mouse IgE mAb
HR1: Histamine receptor 1
IP: Intraperitoneal
IV: Intravenous
MMCP1: Mouse mast cell protease 1
NSGS: NOD/LtSz-SCID IL-2RG$^{-/-}$SGM3 (mice)
OVA: Ovalbumin
PI3K: Phosphoinositide 3-kinase
SC: Subcutaneous
Syk: Spleen tyrosine kinase
TNP: Trinitrophenyl

METHODS

Mice

BALB/c and C57BL/6 mice were either purchased from Charles River Laboratories (Wilmington, Mass) or bred in-house. Human FcεRIα transgenic, mouse FcεRIα-deficient mice on a BALB/c background[43] were a gift from Jean-Pierre Kinet (Cambridge, Mass). NOD/LtSz-SCID IL-2RG$^{-/-}$SGM3 (NSGS)[44] and NRG-SGM3 mice were obtained from James Mulloy (Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio) and bred in-house. These mice were reconstituted with red blood cell–depleted, anti-human CD3 mAb -treated human cord blood cells (mAb OCT-3; BioXCel Therapeutics, New Haven, Conn) as described by Wunderlich et al.[45] Animal work was approved by the Cincinnati Children's Hospital Research Foundation Institutional Animal Care and Use Committee and was conducted in accord with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.[46] Female mice, 8 to 12 weeks old, were used for experiments unless otherwise noted. Cages of mice were randomly assigned to different groups without a specific randomization protocol, with the exception that mice in different groups were age- and sex-matched.

Reagents

Rat IgG_{2a} anti-mouse IgE mAb–secreting hybridoma cells (EM-95)[47] were a gift of Zelig Eshhar (Weizmann Institute, Rehovot, Israel). Mouse IgEαTNP (trinitrophenyl) mAb–secreting hybridoma cells[48] were purchased from the ATCC (Rockville, Md). Mouse IgG_1 anti-human FcεRIα mAb–secreting hybridoma cells (15.1)[49] were a gift of Jean-Pierre Kinet. Mouse IgG_1 anti-human IgD mAb–secreting hybridoma cells were a gift of John Kearny (Birmingham, Ala). Mouse IgG_1 anti-hen egg lysozyme mAb secreting hybridoma cells were a gift of S. Smith-Gill (Bethesda, Md).[50] We purchased mouse IgG_{2b} anti-human FcεRIα mAb AER-37 (also called CRA-1) (BioLegend, San Diego, Calif), doxepin hydrochloride (Sigma, St Louis, Mo), triprolidine hydrochloride (Sigma), formoterol fumarate dihydrate (Sigma), terbutaline sulfate (Akorn Inc, Decatur, Ill), albuterol sulfate (Sigma), indacaterol maleate (Selleckchem, Houston, Tex), adrenaline hydrochloride solution–1 mg/mL (Par Pharmaceutical Cos Ind, Spring Valley, NY), fostamatinib R788 (Selleckchem), imatinib mesylate (Santa Cruz Biotechnology, Santa Cruz, Calif), idelalisib (also known as CAL-101, GS-1101) (Selleckchem), and ibrutinib (ChemieTek, Indianapolis, Ind). TNP-labeled BSA was prepared by dissolving 1 g of BSA in 5 mL of saline, then adding 500 μL of 1 mol/L $NaHCO_3$ buffer, pH 9.6. A 200 mg/mL solution of TNP was made by dissolving 500 μL TNP (picrylsulfonic acid) in 500 μL dimethyl sulfoxide (DMSO). TNP/DMSO was added to the BSA solution while vortexing, and the resulting solution was left overnight in the dark at room temperature. The resulting TNP-BSA was dialyzed 3 times against saline and stored at −80°C. TNP-ovalbumin (TNP-OVA) was made as previously described.[51] Formoterol was dissolved in DMSO before dilution in normal saline. Idelalisib was dissolved at 30 mg/mL in 30% polyethylene glycol 400/0.5% Tween80/5% propylene glycol. Table E1 in this article's Online Repository (available at www.jacionline.org) summarizes the dose ranges tested, routes of administration, timing of administration, and vehicles used for the different agents tested; the same vehicle used to dissolve or suspend an agent was used as the control for that agent.

Determination of IgE expression by peritoneal mast cells

IgE staining of peritoneal mast cells (identified as c-kit$^+$ IL-3R$^+$ B220$^-$ high side scatter cells) was determined by flow cytometry.

IL-4C

Mouse recombinant IL-4 (Peprotech, Rocky Hills, NJ) was mixed with BVD4-1D11.1 rat anti-mouse IL-4 mAb (purified from ascites) at a 1:5 weight ratio and incubated for 2 minutes at room temperature, then diluted to the desired concentration with 1% autologous mouse serum in PBS.

Measurement of MMCP1 levels

Serum levels of mouse mast cell protease 1 (MMCP1) were measured in blood drawn 3 or 4 hours after challenge with an ELISA kit (eBioscience, Thermo Fisher Scientific, Waltham, Mass), according to the manufacturer's protocol.

Anaphylaxis

The severity of anaphylactic shock was assessed by decrease in rectal temperature, as measured by digital thermometry.[52]

Passive anaphylaxis model

Mice were sensitized by intravenous (IV) injection of 10 μg of IgE anti-TNP mAb, then challenged IV 24 hours later with 10 μg of TNP-BSA or TNP-OVA.

Generation of mouse anti-human FcεRIα mAbs

FcεRIα-deficient mice (BALB/c background) were immunized 3 times intraperitoneally (IP) at 2-week intervals with 20 μg of the 176 N-terminal amino acid human FcεRIα ectodomain in 50% alum adjuvant. Once high titers of mouse IgG_1 anti-human FcεRIα were detected by ELISA, the mice were injected IV with 2 μg of human FcεRIα ectodomain; 2 days later, their splenocytes were fused with a nonsecreting mouse plasmacytoma cell and cloned. Five mouse IgG_1 anti-human FcεRIα ectodomain mAb–secreting clones were selected by ELISA, followed by flow cytometric evaluation of their ability to stain peritoneal mast cells from mice that expressed human, but not mouse FcεRIα and their failure to stain peritoneal mast cells from normal BALB/c mice. Selected clones, including the clones IE7 and IB10 that are used in this article, were expanded for mAb generation and grown as ascites in pristane-primed BALB/c mice. IE7 and IB10 mAbs were purified from ascites by ammonium sulfate fractionation followed by DE-52 chromatography.

Statistics

Differences in temperature and concentrations of MMCP1 were compared with a 2- or 3-way ANOVA or Kruskal-Wallis test, as appropriate, followed by Tukey's honest significant difference test or Mann-Whitney *U* test, as appropriate (GraphPad Prism 7.0; GraphPad Software, La Jolla, Calif). A 1-tailed test was used to test hypotheses that a given treatment would decrease the temperature drop; otherwise a 2-tailed test was used. For line graphs showing development of hypothermia, statistical analysis was performed on the maximum temperature drops for individual mice. A P value of $< .05$ was considered significant. For all figures, $*P < .05$; $**P < .01$; $***P < .001$; $****P < .0001$.

RESULTS

Effects of antihistamines

Initial experiments evaluated the ability of single small molecule inhibitors to suppress anaphylaxis in BALB/c mice injected IV with 20 μg of EM-95. In a total of 10 experiments, each of which had 4 to 6 mice per group, 200 μg of HR1 antagonist triprolidine, injected IP 1 hour prior to IV EM-95 challenge, decreased the maximum temperature drop on average from 3.98°C ± 0.23°C to 2.22°C ± 0.25°C (mean ± SEM, 44.9% ± 4.0% suppression), with a typical result shown in Fig 1, *A*. Doses of triprolidine <200 μg were less effective than the 200-μg dose, while doses >200 μg had no additional suppressive effect; triprolidine injection 37 or 75 minutes prior to EM-95 challenge were equally effective, but triprolidine injected 150 minutes prior to challenge was ineffective (see Fig E1 in this article's Online Repository at www.jacionline.org). An HR1 antagonist that also suppresses the HR2 (doxepin,[53] 10 mg/kg), was no more effective than triprolidine at suppressing IgE-mediated hypothermia (Fig 1, *B*); an H2R antagonist (ranitidine) had no effect (Fig 1, *C*) and 25 mg/kg of ketotifen, which is thought to be both an HR1 antagonist and a mast cell stabilizer,[54] was considerably less effective than 200 μg of triprolidine at preventing IgE-mediated hypothermia (not shown).

Effects of β-adrenergic receptor agonists

Because β-adrenergic receptor agonists have been described to suppress both mast cell degranulation[55,56] and the effects of mast cell-generated mediators,[57,58] we tested the ability of drugs in this class to suppress IgE-mediated anaphylaxis; these drugs (formoterol, terbutaline, albuterol, and indacaterol) indeed dose-dependently protected against development of hypothermia (Fig 2). In contrast, subcutaneous injection of 2 to 50 μg of epinephrine 5 minutes after antigen challenge of mice primed with antigen-specific IgE failed to prevent hypothermia (see Fig E2 in this article's Online Repository at www.jacionline.org). IP injection of 2 μg of epinephrine 5 minutes after antigen challenge also failed to reverse the development of hypothermia and IP injection of 10 or 50 μg of epinephrine was toxic, inducing hypothermia even in mice that received no other treatment (not shown). In contrast, subcutaneous (SC) injection of 25 μg of epinephrine 10 minutes prior to anti-IgE mAb challenge significantly decreased development of hypothermia, particularly when results were adjusted to eliminate the hypothermic effects directly induced by epinephrine (see Fig E3, *A* in this article's Online Repository at www.jacionline.org).

Because we had evaluated an HR1 antagonist and β-adrenergic agonists solely as prophylactics (ie, injected prior to anti-IgE mAb challenge) and epinephrine has some effectiveness as a prophylactic, but not as a therapeutic (injected after anti-IgE mAb challenge) in our model, we evaluated whether an HR1 antagonist and/or a β-adrenergic agonist had any efficacy in our model as therapeutics. Results of this experiment (Fig E3, *B*) show significant efficacy, although less than in experiments that used these drugs prophylactically.

Effects of tyrosine kinase inhibitors

Because the tyrosine kinase Syk is involved in the initiation of IgE-mediated mast cell activation,[59] we also tested the ability of a Syk inhibitor (fostamatinib), to block IgE-mediated hypothermia. This drug was consistently effective at a dose of 80 mg/kg, but not at 40 mg/kg (typical results shown in Fig 3, *A*). Unlike triprolidine and β-adrenergic agonists, fostamatinib suppressed mucosal mast cell degranulation, as shown by inhibition of serum levels of MMCP1, a proteolytic enzyme that is released by degranulating mucosal mast cells[60] (Fig 3, *B*). The BTK inhibitor ibrutinib, at a single dose of 25 mg/kg, failed to suppress IgE-mediated hypothermia in BALB/c mice, but almost completely suppressed IgE-mediated hypothermia in huFcεRIα mice, whose chimeric FcεRI is composed of human FcεRIα and mouse FcεRIβ and FcεRIγ[43] and which develop less severe hypothermia and less mast cell degranulation (lower serum MMCP1) than BALB/c mice in response to anti-IgE mAb (Fig 3, *C* and *D*). Ibrutinib significantly decreased the anti-IgE mAb–induced MMCP1 response in both BALB/c and huFcεRIα mice, but fully suppressed this response only in the huFcεRIα mice (Fig 3, *D*). In contrast to Syk and BTK inhibitors, other tyrosine kinase inhibitors by themselves had little or no suppressive effects on hypothermia or on mast cell degranulation at doses that inhibit their targets *in vivo*. These inhibitors included 5 to 80 mg/kg of the phosphoinositide 3-kinase (PI3K) P110δ inhibitor, idelalisib, and 1.25 mg/kg of the Abl/Kit inhibitor, imatinib (see Fig E4 in this article's Online Repository at www.jacionline.org; data not shown).

Lack of effects of additional agents

In addition to these tyrosine kinase antagonists, a large number of other drugs and agents that have been described to inhibit anaphylaxis[61-71] in mice had little or no effect in our model as single agents when used at the concentration, route of administration, and timing specified in the previously described mouse studies. These included an HR4 inhibitor (JNJ-777120, 20 mg/kg), a natural phenol/phytoalexin (resveratrol, 10 mg/kg), a flavonoid polyphenol (quercetin, 50 mg/kg), an antioxidant flavanol (kaempferol, 50 mg/kg), a natural phenol diarylheptanoid (curcumin, 50 mg/kg), corticosteroids, a mast cell stabilizer (cromolyn sodium, a single 300 μg dose), theophylline (5 mg/kg), a 5-lipoxygenase inhibitor (zileuton, 50 mg/kg dissolved in DMSO and inoculated by oral gavage 1 hour and 24 hours prior to challenge), a leukotriene receptor (CysLT1) antagonist, montelukast (6 mg/kg inoculated subcutaneously 1 hour and 24 hours before challenge), a leukotriene D4 receptor antagonist (REV 5901, 250 μg IV diluted in a 1:1000 solution of DMSO in saline 15 minutes prior to challenge), and a platelet-activating factor antagonist (ABT-491, 2 μg IV). In addition to these, serotonin receptor ($5\text{-HT}_{1/2/2a/7}$) antagonists (metergoline (200 μg IP in 1% carboxymethylcellulose 30 minutes prior to challenge) and ketanserin (60 μg IP 30 minutes prior to challenge) were also shown by us previously to fail to inhibit murine IgE-mediated anaphylaxis.[51] The effects of all of the different agents tested are summarized in Table E2 in this article's Online Repository (available at www.jacionline.org).

Additive or synergistic suppressive effects of an antihistamine, β-adrenergic agonists, and a Syk inhibitor

On finding that antihistamines, β-adrenergic agonists, and a Syk inhibitor were the most effective individual inhibitors of IgE-mediated murine anaphylaxis, studies were performed to determine (1) whether combinations of these agents could

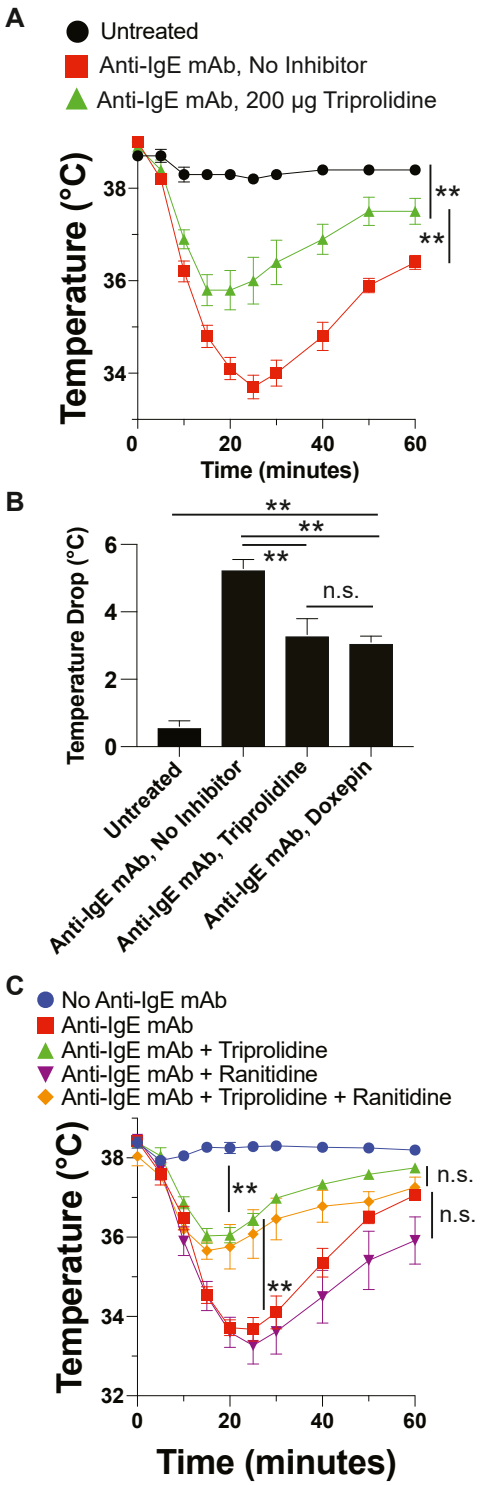


FIG 1. HR1 antagonist antihistamines partially protect against anti-IgE mAb–induced hypothermia. **(A, B)** BALB/c mice (n = 6 per group) were injected IP with 200 μg of triprolidine or vehicle, followed 1 hour later by IV injection of saline (no treatment) or 20 μg of anti-mouse IgE mAb and followed for 60 minutes for development of hypothermia. **(B)** Doxepin (10 mg/kg injected IP 5 minutes before challenge) and triprolidine protect BALB/c mice (n = 6 per group) against anti-IgE mAb–induced hypothermia to the same extent. **(C)** BALB/c mice (n = 5-6 per group) were injected IP with saline, 200 μg of triprolidine, and/or 1 mg/kg of ranitidine, followed 1 hour later by IV injection of saline (no treatment) or 20 μg of anti-mouse IgE mAb and followed for 60 minutes for development of hypothermia. Ranitidine did not significantly suppress IgE-mediated hypothermia; triprolidine + ranitidine did not suppress hypothermia significantly more than triprolidine alone did. Statistical tests: Kruskal-Wallis followed by Mann-Whitney *U* test for all panels. ***P* < .01. *ns*, Not significant.

additively or synergistically suppress anaphylaxis, and (2) whether the use of combinations would allow use of lower doses of a β-adrenergic agonist and a Syk inhibitor, which are more likely than an H1R blocker to have adverse effects. Results of these studies showed additive or synergistic effects of any 2 of these agents (Fig 4). In some experiments, the use of all 3 agents almost totally blocked anaphylaxis (Fig 4, *B*). A similar result was observed in huFcεRIα mice that were challenged with an activating anti-human FcεRIα mAb (see Fig E5, *A* in this article's Online Repository at www.jacionline.org). In other experiments, 3 agents were no more effective than 2 (Fig 4, *D* and *E*). This was true even when mice were pretreated with a long-acting formulation of IL-4[72] to increase anaphylaxis severity[73] (Fig 4, *D*). Although these studies were all performed with female BALB/c mice, the combination of triprolidine and albuterol was also more effective than either drug alone at suppressing IgE-mediated hypothermia in BALB/c male and C57Bl/6 female mice (see Fig E6 in this article's Online Repository at www.jacionline.org).

Additive and synergistic effects of different tyrosine kinase inhibitors

Because Syk, PI3K, and BTK are all involved in FcεRI-mediated mast cell signaling[74] and Kit also promotes mast cell activation,[75] we hypothesized that PI3K, BTK, and Kit inhibitors might act synergistically with a Syk inhibitor (fostamatinib) to suppress FcεRI-mediated mast cell activation, even if they had little or no effect by themselves. This appeared to be the case for each of these inhibitors (Fig 5, *A-C*), although the effects of the combination of fostamatinib and imatinib did not reach statistical significance ($P = .07$) and the combination of fostamatinib with another tyrosine kinase antagonist sometimes caused toxicity (reversible hypothermia) in the absence of FcεRI crosslinking (Fig 5, *B*; data not shown).

The ability of PI3K, BTK, and Kit inhibitors to enhance Syk inhibitor suppression of IgE-mediated anaphylaxis, even though they had little effect on their own in BALB/c mice, led us to evaluate whether the same kinase inhibitors would enhance suppression by an HR1 antagonistic or β-adrenergic receptor agonist in these mice. Results of these studies demonstrated increased inhibition when the BTK antagonist ibrutinib was combined with either triprolidine or albuterol, as compared to triprolidine or albuterol alone, while adding imatinib or idelalisib to triprolidine or albuterol had much less of an effect (Fig 5, *D-F*). The effects of all tested combinations of agents on IgE-mediated anaphylaxis are summarized in Table E3 in this article's Online Repository (available at www.jacionline.org).

Triprolidine, indacaterol, and fostamatinib suppress IgE-mediated anaphylaxis in mice that have human mast cells and basophils

Because human mast cells and basophils have somewhat different properties than the same cell types in mice,[21,76-78] we

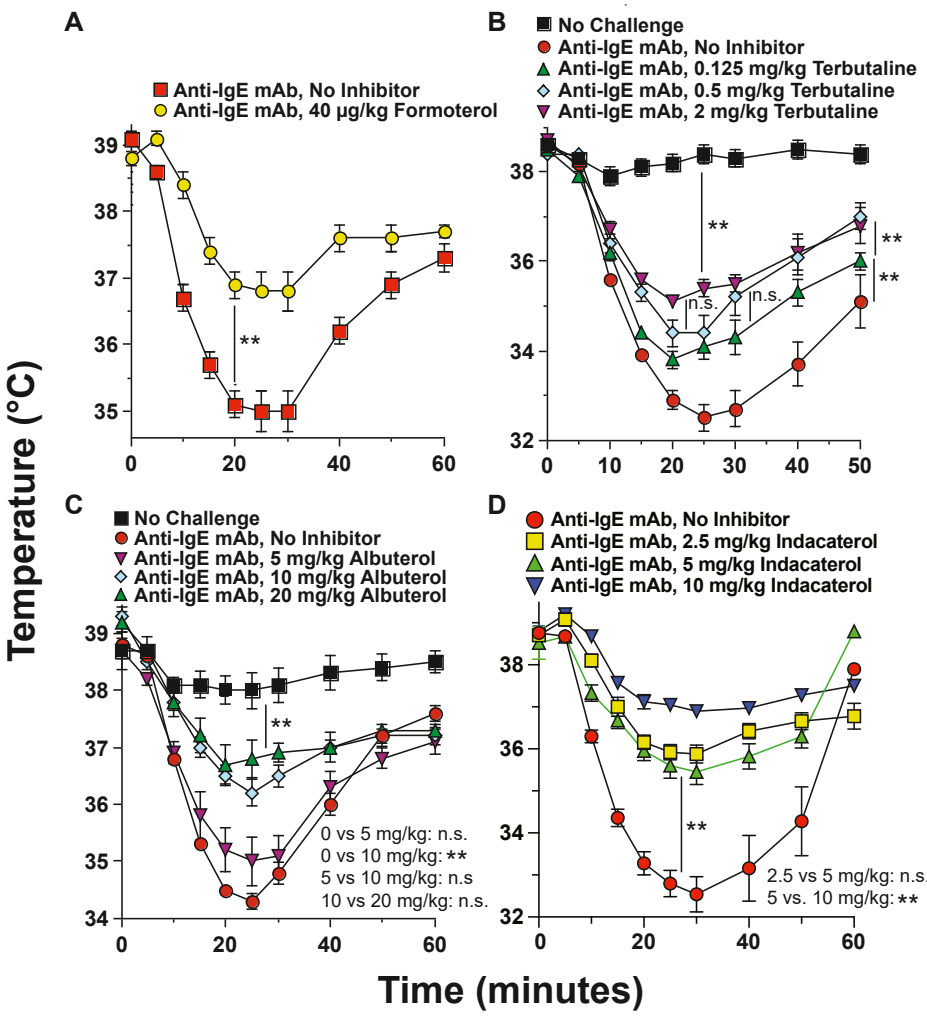


FIG 2. β-adrenergic agonists partially protect against anti-IgE mAb–induced hypothermia. BALB/c mice (n = 4-6 per group) were pretreated with or without formoterol **(A)**, terbutaline **(B)**, albuterol **(C)**, or indacaterol **(D)**; challenged IV with 20 μg of anti-mouse IgE mAb; and followed for 60 minutes for development of hypothermia. Formoterol was injected IV 37 minutes prior to challenge; terbutaline was injected SC 30 minutes prior to challenge; albuterol was injected IP 30 minutes prior to challenge; indacaterol was injected IV 30 minutes prior to challenge. Statistical tests: Kruskal-Wallis followed by Mann-Whitney *U* test for all panels. $^{**}P < .01$.

used immune-deficient, recombinant human (rehu)IL-3–, rehuGM-CSF–, and rehu stem cell factor (SCF)-producing NSGS mice that had been reconstituted for 2 to 3 months with T-cell–depleted human cord blood cells to determine whether antihistamine/β-adrenergic agonist/Syk inhibitor treatment could block IgE-mediated anaphylaxis that is mediated by human mast cells and basophils. These mice provide a particularly sensitive tool for studying IgE-mediated anaphylaxis, because they develop large numbers of human mast cells (along with smaller numbers of basophils); both cell types in these mice have abnormally high responsiveness to FcεRI crosslinking because of their high levels of 3 mast cell–stimulating cytokines,[79,80] which also increase responsiveness to mast cell–produced mediators (Khodoun and Finkelman, unpublished data, 2019). Indeed, IV injection of a relatively high dose of anti-IgE mAb (Fig 6, *A*), antigen following priming with antigen-specific IgE (Fig 6, *B*), or anti-huFcεRIα mAb (Fig 6, *C*) typically kills these mice in <30 minutes, while injection of these mice with 100 to 500 ng of the same anti-FcεRIα mAb typically causes a 4°C to 6°C temperature drop (Fig E5, *B*). Pretreatment with the combination of triprolidine/indacaterol/fostamatinib usually prevented death (Fig 6, *A-C*) and substantially inhibited the development of hypothermia in mice injected with antigen or the higher doses of anti-IgE mAb. Hypothermia was almost completely prevented by the combinations of triprolidine/indacaterol/fostamatinib or triprolidine/indacaterol when mice were challenged IV with the low dose of anti- FcεRIα mAb (Fig E5, *B*).

Fostamatinib, but not ibrutinib, suppresses mast cell/basophil desensitization

Although 80 mg/kg of fostamatinib suppresses anaphylaxis and 40 mg/kg of this Syk inhibitor enhances the abilities of triprolidine and β-adrenergic agonists to suppress anaphylaxis, fostamatinib treatment was associated in some anti-FcεRIα or anti-IgE mAb–challenged mice with a late decrease in

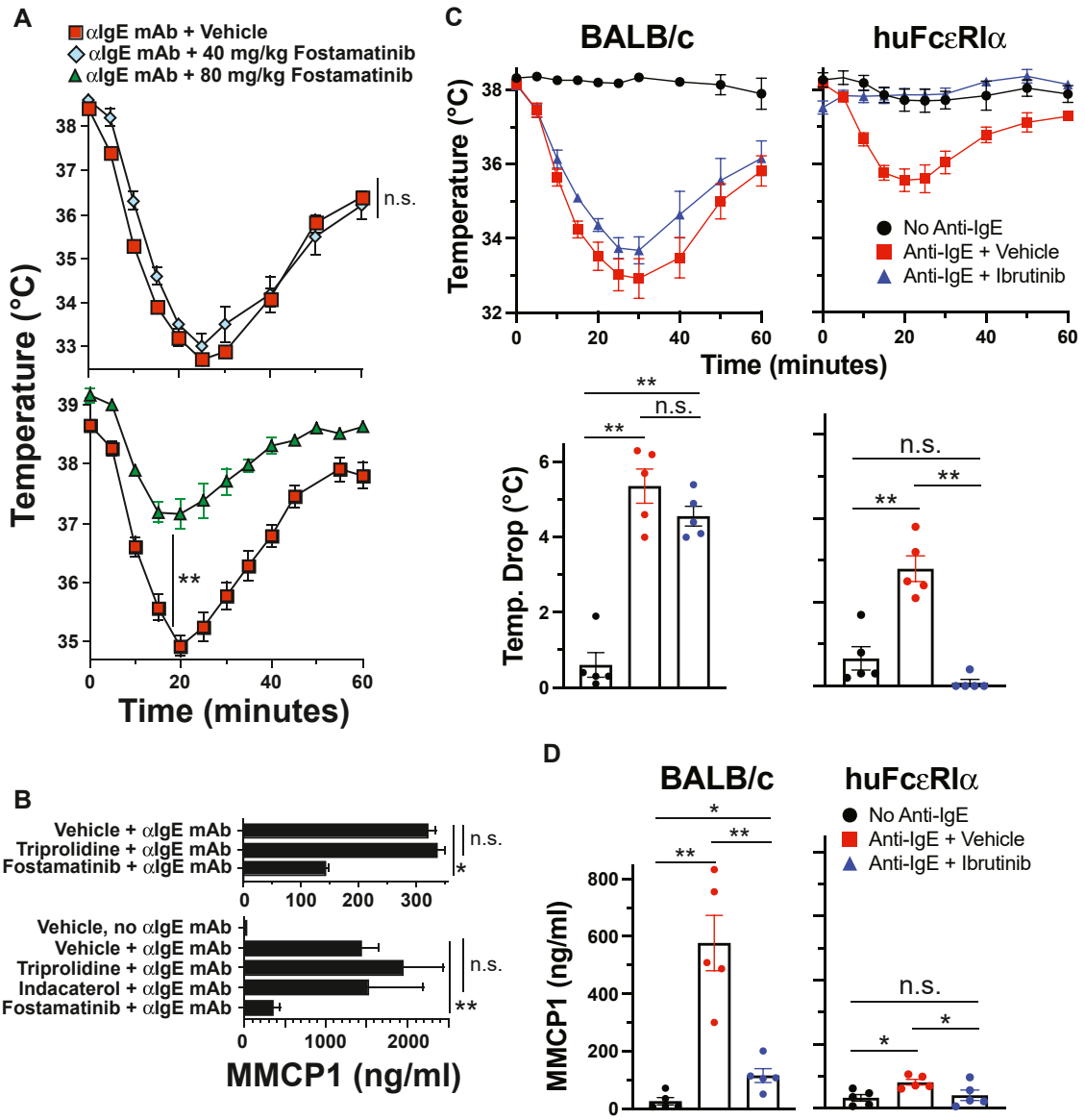
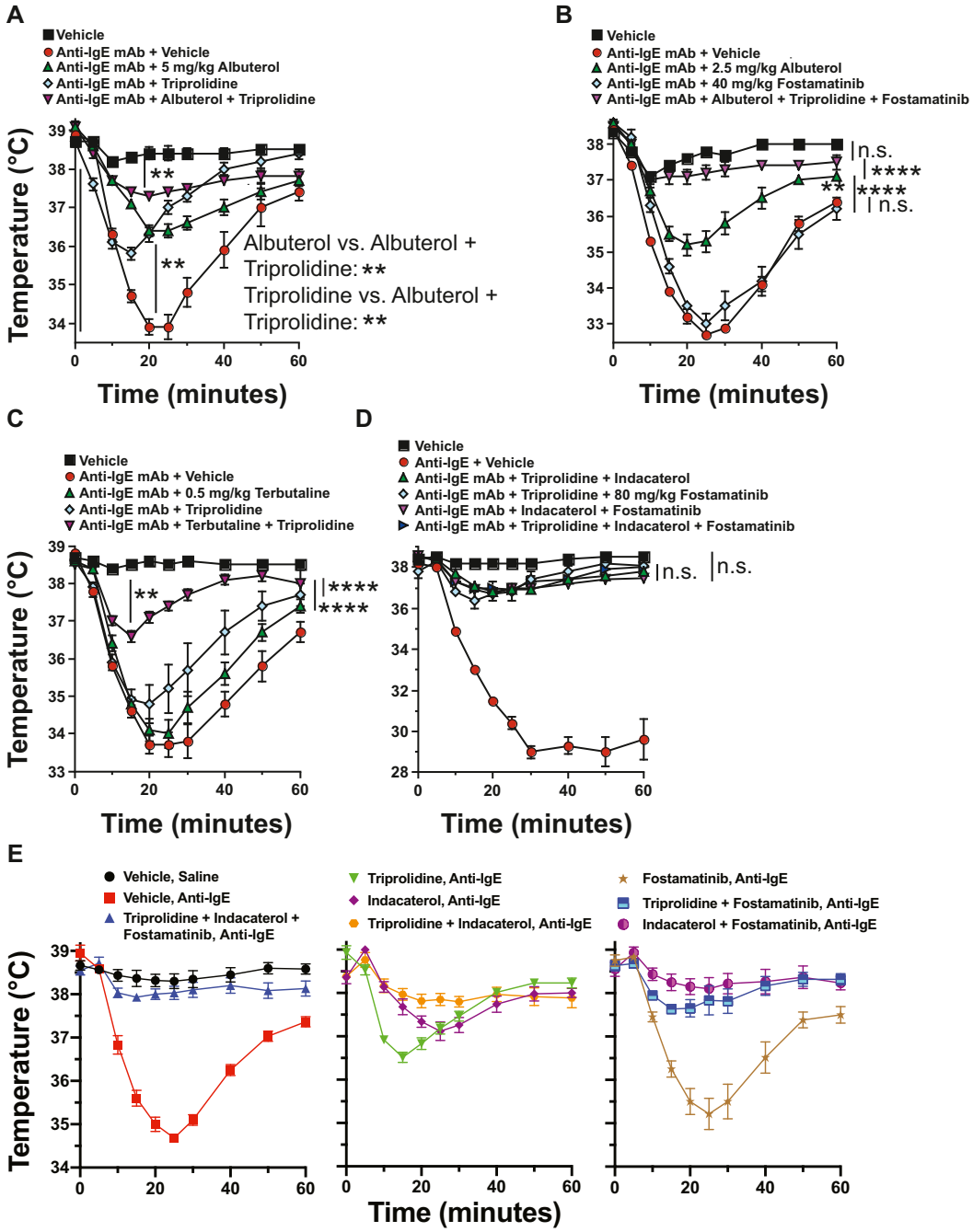


FIG 3. Syk and BTK dependence of anaphylaxis and mast cell degranulation. **(A)** In 2 separate experiments, BALB/c mice (n = 6 per group) were pretreated by IP injection with vehicle or 40 or 80 mg/kg of fostamatinib, then challenged IV with 20 μg of anti-mouse IgE mAb, and followed 60 minutes for hypothermia. **(B)** In 2 separate experiments, BALB/c mice (n = 4-6 per group) were treated 1 hour prior to challenge with vehicle, triprolidine, 80 mg/kg of fostamatinib, or 2.5 mg/kg SC of indacaterol and challenged IV with 20 μg of anti-mouse IgE mAb. Mice were bled 4 hours after challenge and serum MMCP1 was determined by ELISA. **(C)** BALB/c and huFcεRIα mice (n = 5 per group) were pretreated by IP injection with vehicle or 25 mg/kg ibrutinib and challenged 30 minutes later IV with saline or 20 μg of anti-mouse IgE mAb, then followed 60 minutes for hypothermia. Both temperature curves and maximum decreases in temperature are shown. Similar results were observed in a second experiment (not shown). **(D)** In the same experiment shown in **C**, mice were bled 4 hours after anti-IgE mAb or saline challenge and serum MMCP1 levels were determined by ELISA. Statistical tests: **(A)** Mann-Whitney *U* test. **(B-D)** Kruskal-Wallis followed by Mann-Whitney *U* test. **P* < .05; ** *P* < .01.

temperature that occurred once the effects of this drug had worn off (Fig 6, *C*). Because this late exacerbation of hypothermia was not seen in mice that did not receive a Syk inhibitor (data not shown), this result raised the possibility that Syk inhibition might interfere with FcεRI-mediated mast cell desensitization. To evaluate this possibility, huFcεRIα transgenic mice were sensitized with IgE anti-TNP mAb, then treated the next day with anti-huFcεRIα mAb or isotype-control mAb in the presence or absence of 40 mg/kg of fostamatinib. These mice were challenged 4 hours after that with 10 μg of TNP-BSA and evaluated for the development of hypothermia (Fig 7, *A*). Hypothermia failed to develop in the anti-FcεRIα mAb–treated mice that had not received fostamatinib, but hypothermia was only partially suppressed in those that had received this drug (Fig 7, *B*). Fostamatinib had no significant effect on the ~7-fold decrease in mast cell IgE expression that was caused by anti-FcεRIα mAb treatment



Vehicle, Saline vs. Anti-IgE + Triprolidine + Indacaterol: n.s.
Vehicle, Saline vs. Anti-IgE + Triprolidine + Fostamatinib: ns
Vehicle, Saline vs. Anti-IgE + Indacaterol + Fostamatinib: n.s.
Vehicle, Saline vs. Anti-IgE + Triprolidine + Fostamatinib + Indacaterol: n.s.
Anti-IgE + any 2 drugs vs. Anti-IgE + 3 drugs: n.s.
Anti-IgE + Indacaterol vs. Anti-IgE + Indacaterol + Fostamatinib: .0023
Anti-IgE + Indacaterol vs. Anti-IgE + Indacaterol + Triprolidine: .036
Anti-IgE + Triprolidine vs. Anti-IgE + Triprolidine + Indacaterol: .0005
Anti-IgE + Triprolidine vs. Anti-IgE + Triprolidine + Fostamatinib: .0032
Anti-IgE + Fostamatinib vs. Anti-IgE + Indacaterol + Fostamatinib: <.0001
Anti-IgE + Fostamatinib vs. Anti-IgE + Triprolidine + Fostamatinib: <.0001

FIG 4. Inhibition of IgE-mediated anaphylaxis with combinations of an antihistamine, a β-adrenergic agonist, and a Syk inhibitor. **A-E,** BALB/c mice (n = 5-6 per group) were pretreated with the agents shown and challenged IV with 20 μg of anti-mouse IgE mAb, then followed for 60 minutes for development of hypothermia. All mice were treated with IL-4C that contained 1 μg of IL-4 24 hours prior to challenge for experiments shown in **D**. Statistical tests: Kruskal-Wallis followed by Mann-Whitney *U* test for all panels. $^{**}P < .01$; $^{****}P < .0001$.

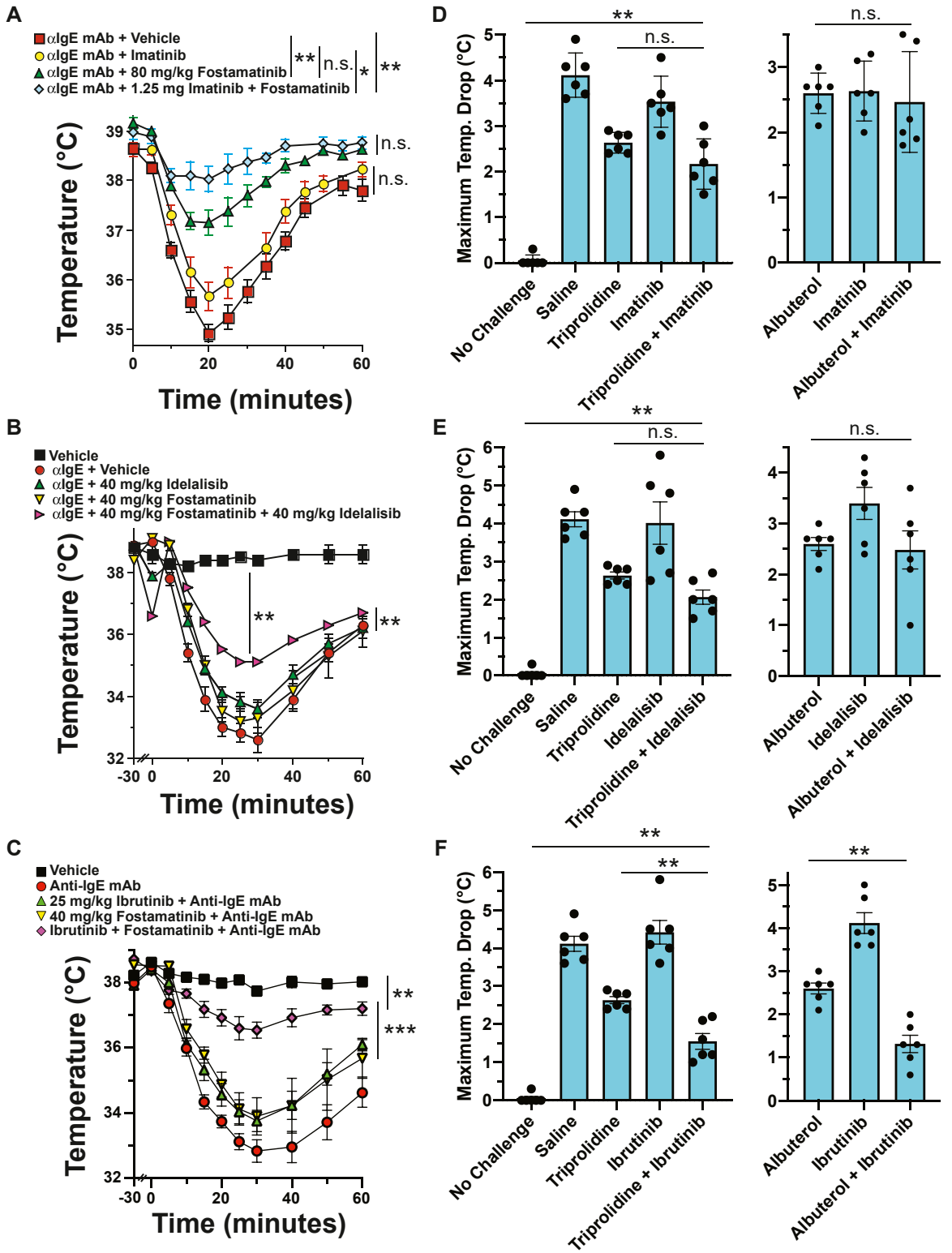


FIG 5. Synergistic inhibition of IgE-mediated anaphylaxis by tyrosine kinase inhibitors. BALB/c mice (n = 4-6 per group) were pretreated IP 30 minutes prior to challenge with vehicle, fostamatinib, imatinib, idelalisib, or ibrutinib, or combinations of fostamatinib with each of the other tyrosine kinase inhibitors, then challenged IV with 20 μg of anti-IgE mAb and followed for 60 minutes for development of hypothermia. Statistical tests: **(A)** Kruskal-Wallis and Mann-Whitney *U* (2-tailed) tests. **(B, C)** Two-way ANOVA and Tukey's honest significant difference test. **(D-F)** Kruskal-Wallis and Mann-Whitney *U* (1-tail) tests with adjustment for multiple groups. $*P < .05$; $**P < .01$; $***P < .001$.

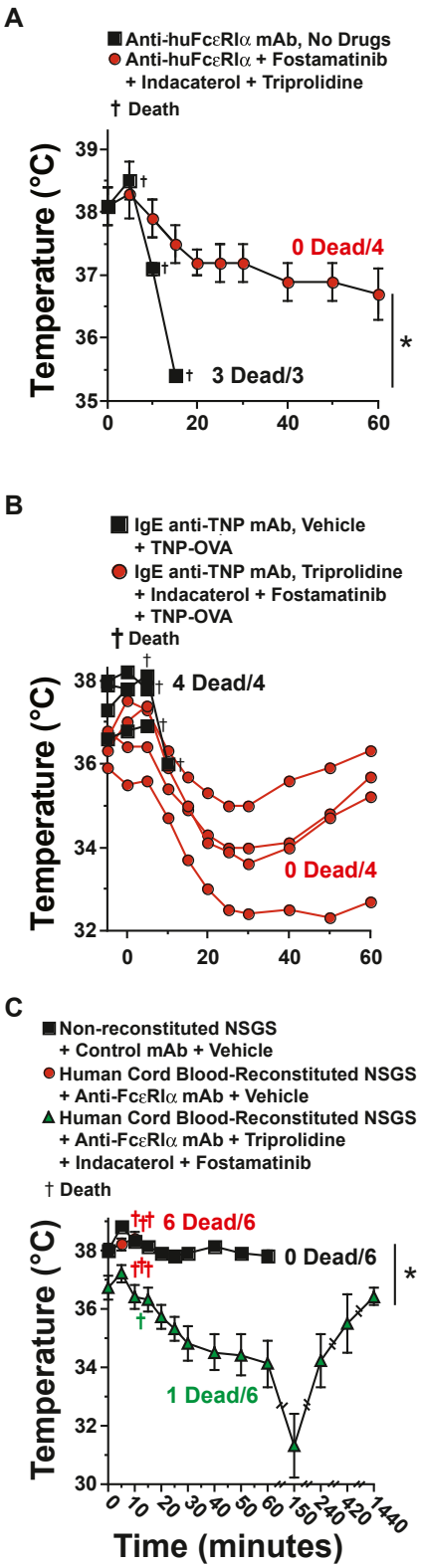


FIG 6. Inhibition of IgE/FcεRI-mediated anaphylaxis in humanized mice by a combination of an antihistamine, a β-adrenergic agonist, and a Syk inhibitor. Nonreconstituted **(C)** or human cord blood–reconstituted **(A-C)** NSGS mice (n = 3-4 per group) were **(A)** pretreated with vehicle (no drugs) or a combination of 80 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol, and 200 μg of triprolidine and challenged IV with 10 μg of an anti-huFcεRIα mAb; **(B)** primed IV with 10 μg mouse IgE anti-TNP mAb, pretreated with vehicle or a combination of 80 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol, and 200 μg of triprolidine and challenged IV with 10 μg of TNP-OVA; or **(C)** pretreated with vehicle or 40 mg/kg of fostamatinib, 2.5 mg/kg of indacaterol, and 200 μg of triprolidine and challenged IV with 50 μg of an anti-huFcεRIα mAb. Statistical tests: Fisher's exact test for comparison of mortality for all panels. *$P < .05$.

(Fig 7, *C*). Thus, Syk inhibition appears to inhibit mast cell desensitization *in vivo*. In contrast, inhibition of BTK, which is also involved in induction of mast cell degranulation, had little or no suppressive effect on IgE-mediated anaphylaxis (hypothermia) in our model (Fig 7, *D* and *E*). (The difference in timing between the fostamatinib and ibrutinib studies was necessary to allow the serum ibrutinib concentration to decrease to a level that would no longer directly suppress anaphylaxis in huFcεRIα mice.)

DISCUSSION

The considerable and increasing prevalence of IgE-mediated allergic disorders, including drug allergy, food allergy, venom allergy, and chronic urticaria, has encouraged the development of safe and effective therapies for these disorders. Rapid desensitization is a therapeutic approach that has been increasingly used, predominantly to treat drug allergy, but also to some extent as part of strategy to treat food allergy. In this approach, patients with allergy are administered rapidly increasing quantities of the relevant allergen or allergen-containing substance, starting with a dose that is insufficient to elicit clinically apparent signs, symptoms, and basophil/mast cell degranulation, and ending with a dose that is hopefully sufficient to temporarily desensitize these cells to exposure to a fully therapeutic dose of a drug or to typically ingested quantities of a food. We have extended this approach by demonstrating that rapid desensitization can be applied in a "polyclonal," antigen-nonspecific way in mice by treating them with serially increasing doses of a mAb to the IgE-binding chain of the basophil and mast cell high affinity IgE receptor, FcεRIα.[18,81,82] In addition to its ability to desensitize to all IgE-mediated anaphylactic reactions, this approach has safety and efficacy advantages over desensitization with antigen, an advantage that most likely reflects the lack of preexisting IgG antibodies to anti-FcεRIα mAb and the longer *in vivo* half-life of IgG antibodies than most allergens. At least 2 mechanisms are involved in mast cell desensitization in our model: suppression of FcεRI signaling and depletion of mast cell/basophil IgE and FcεRI.[18]

The use of rapid desensitization with either antigen or anti-FcεRIα mAb is not without risk, because excessive crosslinking of FcεRI by either agent can cause the same IgE-mediated reactions that the approach is designed to prevent. Indeed, such reactions have occurred frequently during rapid desensitization for drug allergy and can be sufficiently severe to require epinephrine injection.[83,84] For this reason, allergy researchers, particularly the group headed by Marianna Castells, have used combinations of drugs to suppress allergic reactions that can occur during rapid drug desensitization.[15,36-39,42] These drugs, which include HR1- and HR2-specific antihistamines, aspirin, the leukotriene antagonist montelukast, adrenocorticosteroids, and opioids, provide some protection against frequently encountered adverse reactions, including pruritis, urticaria, bronchospasm, angioedema, flushing, and fever. However, there has not been a systematic evaluation of potentially protective agents in an animal model of

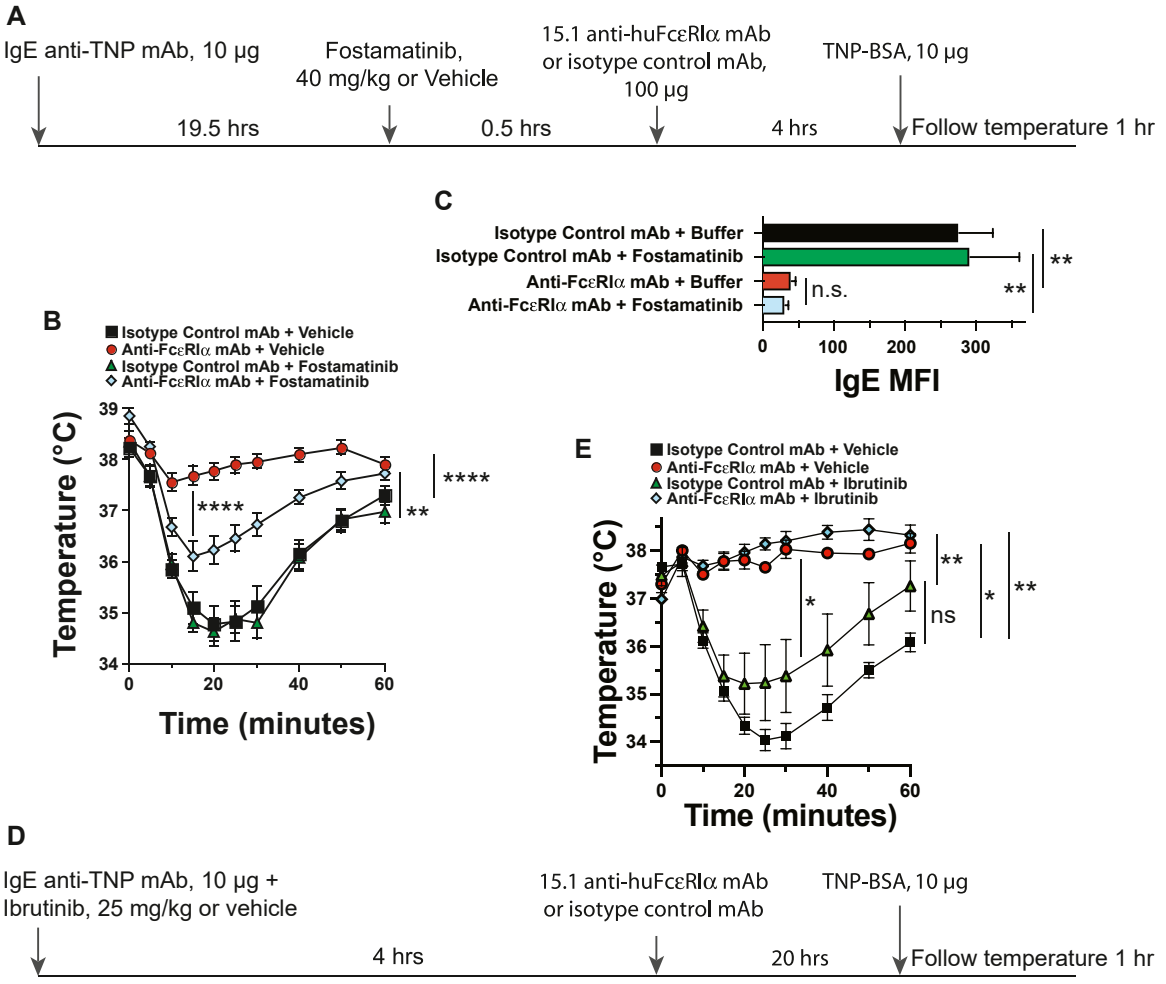


FIG 7. Fostamatinib inhibits mast cells and/or basophil desensitization. **(A, B)** In 2 pooled experiments, a total of 12 huFcεRIα transgenic mice per group were sensitized IV with 10 μg of mouse IgE anti-TNP mAb (which binds avidly to huFcεRIα), and pretreated the next day with 40 mg/kg of fostamatinib or vehicle, followed 30 minutes later by IV injection of 100 μg of 15.1 anti-huFcεRIα mAb or isotype control mAb. Four hours later, mice were challenged IV with 10 μg of TNP-BSA and followed for 60 minutes for development of hypothermia. **(C)** In a separate experiment (n = 5 mice per group), peritoneal mast cells were analyzed for membrane expression of mouse IgE by flow cytometry 24 hours after anti-FcεRIα mAb injection. Fostamatinib was used at 80 mg/kg. **(D, E)** The ability of ibrutinib to suppress mast cell desensitization *in vivo* by 15.1 anti-FcεRIα mAb was tested, using the diagrammed protocol. n = 6 mice per group. Statistical tests: **(A)** Kruskal-Wallis followed by Mann-Whitney *U* test. **(B)** Kruskal-Wallis and 1-tailed Mann-Whitney *U* test with correction for multiple comparisons. **P* < .05; ***P* < .01; *****P* < .0001. *MFI*, Mean fluorescence intensity.

anaphylaxis that could be thoroughly studied and rigorously controlled, and there have not been any reports of drug prophylaxis against allergic reactions to anti-FcεRIα mAbs. Consequently, we undertook the mouse studies that are described in this article.

Using (1) conventional mice and humanized mice that express human FcεRIα on mouse mast cells and basophils or that have human mast cells and basophils; (2) high dose anti-IgE mAbs, anti-FcεRIα mAbs, or IgE anti-TNP mAbs followed by TNP-OVA or TNP-BSA to trigger anaphylaxis; and (3) shock (measured as hypothermia) as a readout for anaphylaxis, we identified agents that were effective by themselves and in combination, agents that were effective only in combination, and agents for which we found no evidence of efficacy. The first, most efficacious group, included HR1-specific antihistamines, β-adrenergic agonists, and an inhibitor of the tyrosine kinase, Syk. In addition to their abilities to partially suppress anaphylaxis by themselves, our results demonstrate additive and synergistic effects that completely or nearly completely prevent hypothermia. This most likely reflects the different mechanisms of action of these 3 therapeutics: blocking the effect of mast cell/basophil-released histamine on HR1; β-adrenergic suppression of increases in vascular permeability through direct effects on vascular endothelial cells;[85] and inhibition of mast cell degranulation by blocking a tyrosine kinase, Syk, that has a critical role in this process (notably, the Syk inhibitor is the only one of these agents that suppresses mast cell degranulation, as evaluated by serum MMCP1 levels). Importantly, a considerable increase in efficacy was seen when any 2 agents were combined and treatment with drug combinations allowed the use of lower doses of individual drugs, which

should decrease drug toxicity. Although an HR1 antagonist and β-adrenergic agonist had some ability to decrease the loss in core body temperature when injected 5 minutes after anti-IgE mAb challenge, this effect was considerably less than when these drugs were used prophylactically.

Agents in the second group had little or no efficacy by themselves at the doses used (higher doses were toxic) in BALB/c mice, but they amplified the protective effect of Syk inhibition. These agents include 3 tyrosine kinase antagonists: imatinib, which suppresses Kit (required for mast cell development and survival[86]), idelalisib, which suppresses P110δ (the δ isoform of PI3K, which is involved in FcεRI signaling[87,88]), and ibrutinib, which suppresses BTK (also important in FcεRI signaling[89]). Thus, the combined use of tyrosine kinase inhibitors that suppress FcεRI signaling at different stages appears to synergistically inhibit FcεRI-mediated mast cell degranulation, just as suppressing different steps in anaphylaxis pathogenesis (mast cell degranulation, histamine binding to the HR1, mediator-induced increases in vascular permeability) additively or synergistically suppresses anaphylaxis. Ibrutinib was unique among these tyrosine kinase antagonists in acting synergistically with an HR1 inhibitor and a β-adrenergic receptor agonist to suppress IgE-mediated anaphylaxis. Unlike Syk activation, the downstream activation of BTK is not an absolute requirement for mast cell degranulation because of the presence of alternative pathways. The importance of the BTK pathway becomes apparent in our model, however, when other inhibitors prevent compensation for a delay or moderate decrease in mast cell degranulation and in mice that express a chimeric FcεRI that appears to signal less potently than wild-type FcεRI does.

A second-generation BTK inhibitor, acalabrutinib, has recently been shown by Dispenza et al[90] to suppress antigen-induced, IgE-mediated anaphylaxis in passively sensitized human cord blood–reconstituted immunodeficient mice, which produce human mast cells, although this suppression can be overcome by increasing the dose of the challenge antigen. Potential differences in the effectiveness of acalabrutinib versus ibrutinib at suppressing BTK, potential differences in the susceptibility of human versus mouse BTK to suppression by these drugs, potential differences in the potency of antigen versus anti-IgE mAb at inducing the BTK-independent pathway of mast cell degranulation, and the longer period of treatment of mice prior to antigen challenge by Dispenza et al[90] than in our study might explain the more effective BTK inhibitor suppression of mast cell degranulation in the Dispenza study than in this article. Regardless of mechanism, the ability of BTK inhibitors to partially suppress mast cell degranulation by themselves and to synergize with inhibitors that work through different mechanisms, without inhibiting mast cell desensitization, suggests a potential for prophylactic use during desensitization. However, ibrutinib is considerably more expensive than HR1 antagonists and β-adrenergic agonists, and it is not yet known whether it can enhance the suppressive effect of an HR1 antagonist/β-adrenergic receptor agonist combination.

The third group of agents tested had little or no efficacy at suppressing anti-FcεRIα mAb– or anti-IgE mAb–induced hypothermia by themselves or when combined with an HR1-specific antihistamine. This group includes HR2 and HR4 inhibitors, cromolyn, theophylline, zileuton, montelukast, a platelet-activating factor antagonist, a bradykinin receptor 2 antagonist, an inhibitor of plasma kallikrein, and serotonin receptor antagonists, in addition to a number of nutraceuticals that have been described by others[61-71] to suppress anaphylaxis in mouse models. Surprisingly, epinephrine was without efficacy as a therapeutic in our model, although it had some efficacy as a prophylactic. At lower doses, epinephrine had no obvious toxic effects but failed to alter hypothermia development when injected after anti-IgE mAb challenge, while higher doses induced hypothermia by themselves, most likely by activating β-adrenergic receptors sufficiently to decrease cardiac output by increasing arterial resistance. This possible explanation is consistent with the greater effect of α-adrenergic stimulation in mice than in humans. Although epinephrine's α-adrenergic receptor–mediated vasoconstriction is thought to increase recovery from human anaphylaxis by enhancing its β-adrenergic receptor-mediated increases in heart rate and contractility, the α-adrenergic receptor–related adverse effects of this drug make it unsuitable for prophylactic use.[91,92]

The most important practical consequence of our work is evidence that adding a nontoxic dose of a β-adrenergic agonist to an HR1-specific antihistamine provides considerably better protection against FcεRI-mediated anaphylaxis than the HR1-specific antihistamine alone. Several β-adrenergic agonists are US Food and Drug Administration–approved drugs, and these drugs are easily available, relatively inexpensive, and have been used for many years to treat asthma. Consequently, their use with antihistamines for prophylaxis during rapid desensitization seems reasonable and practical. In contrast, the clinical use of a Syk inhibitor, such as fostamatinib, as prophylaxis during rapid desensitization may be problematic. Fostamatinib, while approved for use in immune thrombocytopenic purpura, is expensive and appears to block mast cell desensitization. The last issue might not be a problem for very short-term suppression of anaphylaxis, but it would likely be problematic for use during rapid desensitization, which depends, at least in part, on temporary inhibition of mast cell signaling.[18] In contrast to our *in vivo* observation, previous *in vitro* studies found that FcεRI-mediated mast cell and basophil desensitization was not blocked by a Syk inhibitor.[93] This apparent discrepancy probably represents an *in vivo/in vitro* difference, although the possibility that it reflects the use of different Syk inhibitors in the *in vitro* and *in vivo* studies cannot be excluded (the inhibitor that was used for the *in vitro* studies is no longer available).

One strength of our article is its evidence that prophylaxis with 2 or 3 drugs has similar suppressive effects on FcεRI-mediated anaphylaxis in human cord blood–reconstituted NSGS and NRG-SGM3 mice, which have human mast cells and basophils, as it has in normal mice. Substantial inhibition (albeit incomplete) was observed in these reconstituted mice even though transgenic production of human stem cell factor, IL-3, and GM-CSF in this model causes the production of a large number of human mast cells, partially activates these cells, and increases sensitivity to histamine.

Our observations, however, have 4 important limitations. First, agents that have little or no ability to inhibit mast cell degranulation in mice may have considerable ability to inhibit mast cell degranulation in other species, including humans. In this regard, cromolyn has been shown to have greater ability to suppress mast cell degranulation in rats than in mice.[94] Second, even mice that have human mast cells have mouse, rather than human tissues that respond to mast cell–released mediators; these may respond differently than human tissues do. Our failure to observe a therapeutic effect of epinephrine may be an example

of this. Third, our interpretations about the mechanisms responsible for the effects of some of our inhibitors are complicated by the incomplete specificity of some of these inhibitors. For example, fostamatinib inhibits some tyrosine kinases, including the src-family kinases, JAK1, JAK3, c-Kit, and Flt 3 in addition to Syk.[95] Consequently, we cannot totally eliminate the possibility that these off-target effects of fostamatinib contribute to its inhibition of mast cell degranulation and/or mast cell desensitization. We think this unlikely, however, because fostamatinib inhibits Syk 5 times more potently than it inhibits the other tyrosine kinases when studied *in vitro* on mouse mast cells,[59] while a fostamatinib dose (40 mg/kg) that is barely able to inhibit Syk-dependent mast cell degranulation *in vivo* significantly suppresses mast cell desensitization (Fig 7, *A-C*). Fourth, our murine models, even those with human mast cells and basophils, do not develop detectable IgE-mediated disease other than the development of shock (eg, urticaria, bronchospasm, flushing, angioedema, and fever). Consequently, drugs that suppress these disease features but do not suppress shock (which is predominantly mediated in mice by vascular leak) will not be found to be efficacious in our models. This may explain why some of the drugs found useful by Castells and her colleagues for IgE-mediated features other than shock, including flushing and bronchospasm,[42] had no efficacy in our model. Thus, our observation that β-adrenergic agonists are useful for preventing shock during rapid desensitization is more likely to be human-relevant than the failure of several other agents to ameliorate anaphylaxis in our models. We look forward to clinical trials that evaluate the usefulness of adding relatively small doses of β-adrenergic agonists to antihistamines during rapid drug desensitization and that evaluate rapid desensitization with anti-FcεRIα mAbs.

Clinical implications: The combined prophylactic use of an HR1-specific antihistamine and a β-adrenergic receptor agonist can increase the safety of rapid desensitization.

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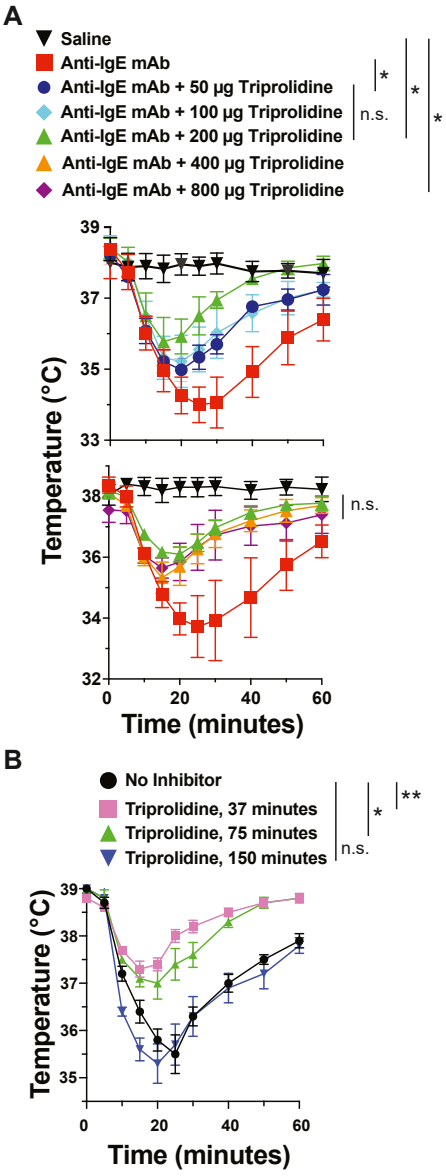

FIG E1. **(A)** Triprolidine dose-response (n = 4-5 mice per group) and **(B)** kinetic data (n = 4-5 mice per group) establish that the maximum ability of this antihistamine to inhibit IgE-mediated anaphylaxis is achieved by administration of at least 200 µg/mouse 30 to 60 minutes prior to challenge. Statistical tests: Kruskal-Wallis and 1-tailed Mann-Whitney *U* test with correction for multiple comparisons for both panels.

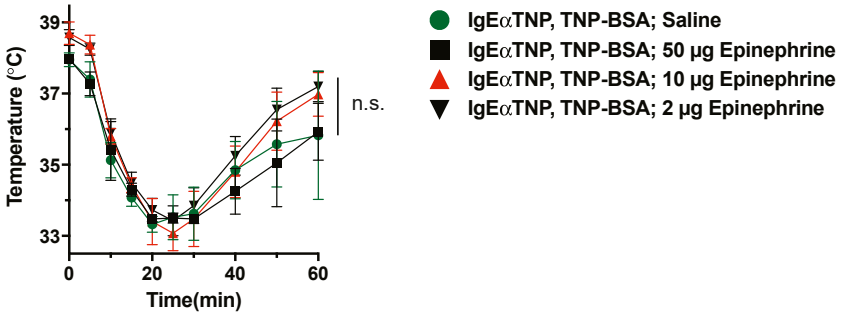

FIG E2. Epinephrine fails to inhibit the development of IgE-mediated anaphylaxis in BALB/c mice. BALB/c mice (n = 4 per group) were primed by injection of 10 μg of IgE anti-TNP mAb and challenged IV the next day with 10 μg of TNP-BSA. Five minutes after challenge mice were injected SC with saline or 2, 10, or 50 μg of epinephrine and followed for 1 hour for development of hypothermia. No significant differences were found using Kruskal-Wallis test.

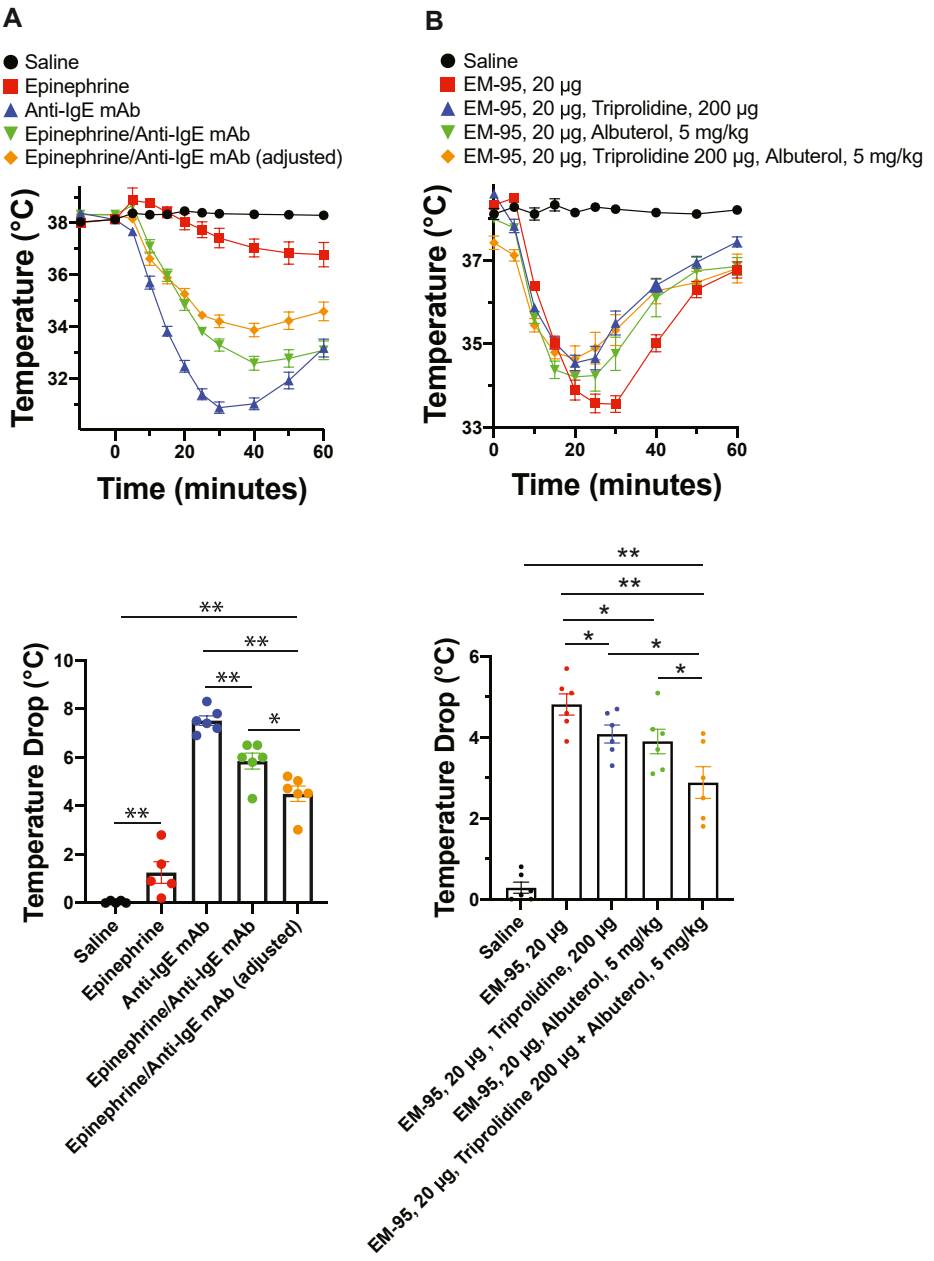

FIG E3. Prophylactic effect of epinephrine and therapeutic effects of triprolidine and albuterol on IgE-mediated anaphylaxis. **(A)** BALB/c mice (n = 5-6 per group) were injected SC with saline or 25 µg of epinephrine. Ten minutes later they were injected IV with saline or 20 µg of EM-95 mouse anti-IgE mAb and rectal temperatures were followed for 60 minutes. To adjust for the direct effects of epinephrine on rectal temperature, deviations from baseline in mice injected with epinephrine alone *(red line)* were subtracted for each time point from temperatures in mice injected with epinephrine + anti-IgE mAb *(green line)*. Adjusted temperatures are shown on the *orange line*. **(B)** BALB/c mice (n = 6 per group) were injected IV with saline or 20 µg of EM-95 anti-mouse IgE mAb. Five minutes later some mice were injected IP with the doses of albuterol and/or triprolidine shown. Rectal temperatures were followed for 60 minutes after anti-mouse IgE mAb injection. Statistical tests: Kruskal-Wallis and Mann-Whitney *U* tests for both panels. **P* < .05; ***P* < .01.

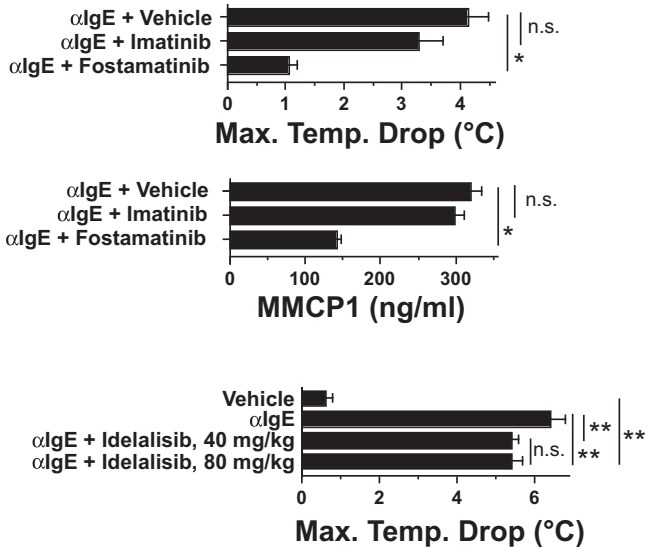

FIG E4. Fostamatinib, but not imatinib, idelalisib, or ibrutinib, provides acute protection against anaphylaxis in BALB/c mice. BALB/c mice (n = 4-6 per group) were pretreated with vehicle, imatinib (1.25 mg/kg), fostamatinib (80 mg/kg), idelalisib, or ibrutinib (10 mg/kg) and challenged IV with 20 μg of anti-mouse IgE mAb. Mice were followed for 60 minutes for development of hypothermia; sera were obtained from some mice 4 hours after challenge and MMCP1 levels were determined by ELISA. Statistical tests: 1-way ANOVA with 1-tailed Mann-Whitney *U* test with correction for multiple comparisons. **P* < .05; ***P* < .01.

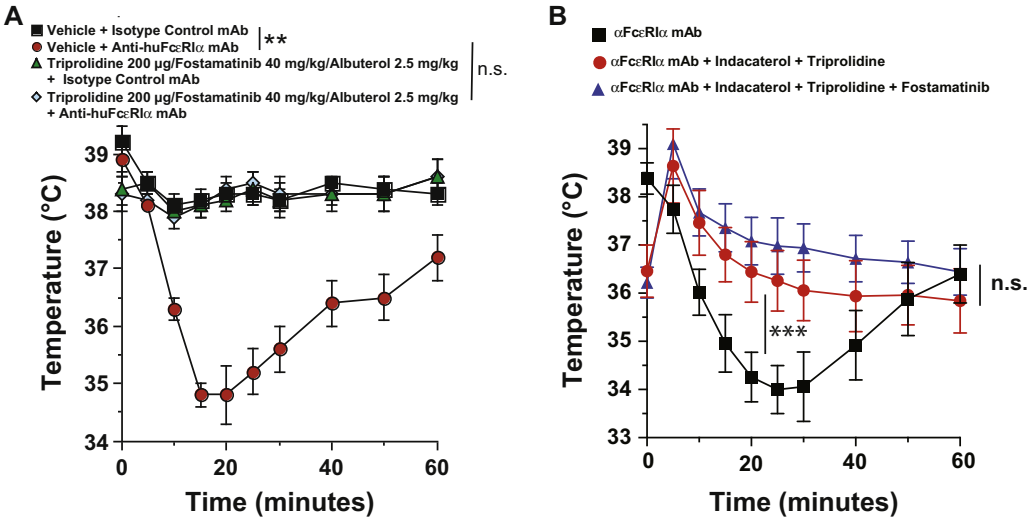

FIG E5. Combined antihistamine (triprolidine), β-adrenergic agonist (albuterol), and Syk inhibitor (fostamatinib) treatment protects highly susceptible mice from FcεRI-mediated anaphylaxis. **(A)** huFcεRIα transgenic mice (n = 5 per group) were pretreated with vehicle or a combination of triprolidine, albuterol, and fostamatinib and challenged IV with 50 μg of AER-37 anti-huFcεRIα or isotype-control mAb, then followed for 60 minutes for development of hypothermia. Statistical tests: Kruskal-Wallis and 1-tailed Mann-Whitney *U* test with correction for multiple comparisons for both. **(B)** reNSGS mice were pretreated with vehicle, 2.5 mg/kg of indacaterol + 200 μg of triprolidine; or indacaterol, triprolidine + 40 mg/kg of fostamatinib and challenged IV with 500 ng of anti-huFcεRIα mAb. Mice were followed for 60 minutes for rectal temperature. Statistical tests: **(A)** 2- or 3-way ANOVA and Tukey's honest significant difference test. **(B)** 3-way ANOVA and Tukey's honest significant difference test. **P* < .05; ***P* < .01; ****P* <.001; *****P* < .0001.

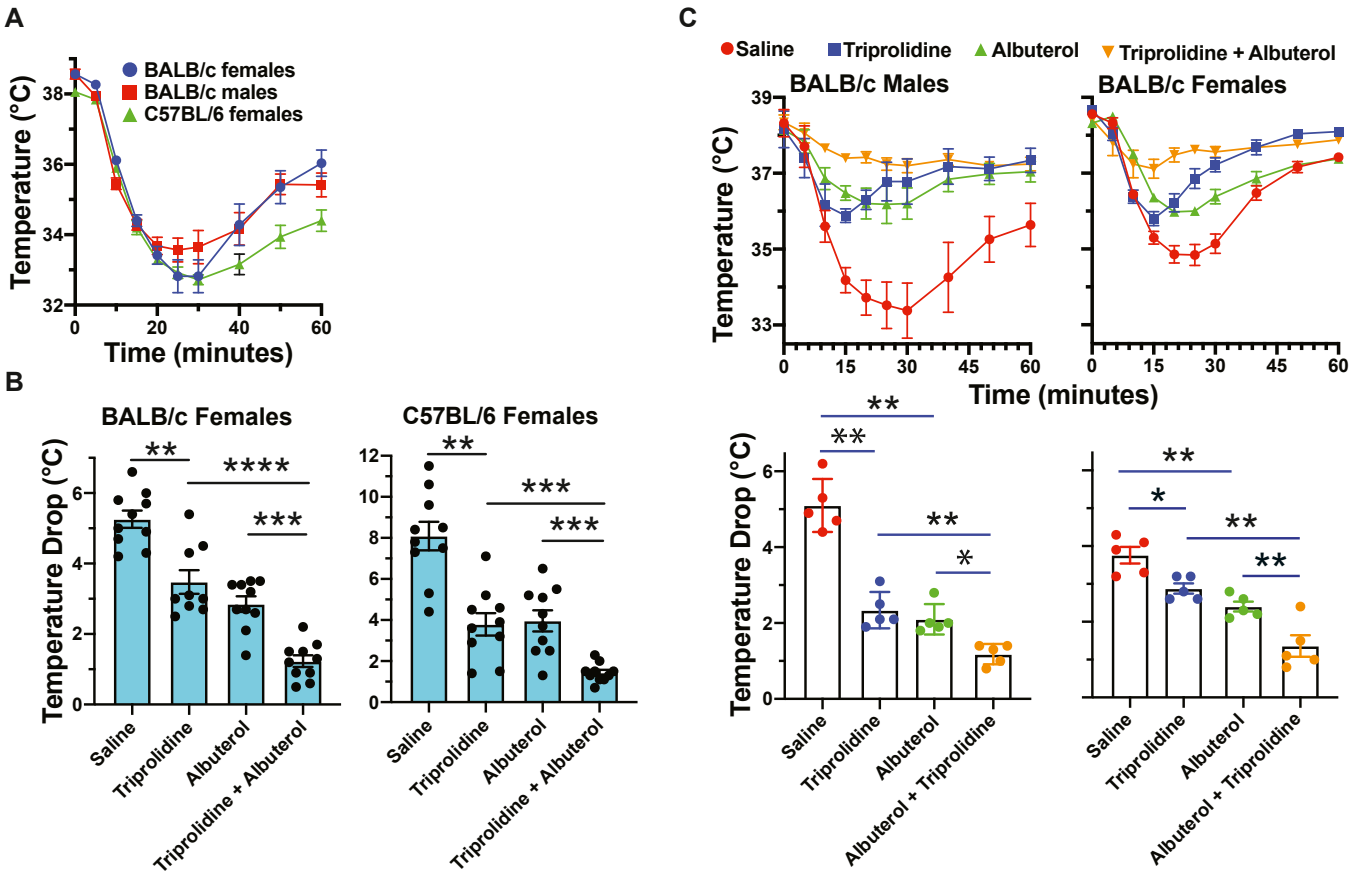

FIG E6. The combination of albuterol and triprolidine enhances protection against IgE-mediated anaphylaxis in male and female BALB/c mice and female C57Bl/6 mice. **(A)** Anti-mouse IgE mAb induces anaphylaxis in male and female BALB/c mice and female C57Bl/6 mice. **(B)** The combination of albuterol 5 mg/kg IP and triprolidine 200 μg IP decreases hypothermia induced by 20 μg EM-95 anti-IgE mAb in both BALB/c and C57Bl/6 female mice. Combination of 2 experiments, total of 10 mice per group. **(C)** The combination of albuterol 5 mg/kg IP and triprolidine 200 μg IP decreases hypothermia induced by 20 μg of EM-95 anti-IgE mAb in both BALB/c male and female mice. n = 5 mice per group. Statistical tests: Kruskal-Wallis and 1-tailed Mann-Whitney *U* test with correction for multiple comparisons for all panels. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

TABLE E1. Agents tested for inhibition of IgE-mediated anaphylaxis

Agent	Dose range	Route	Minutes prechallenge	Vehicle
Fostamatinib	2.5, 5, 10, 20, 40, or 80 mg/kg	IP	30, 37, 60, 75, 120, 150	0.1% carboxymethylcellulose
Imatinib	1.25 mg	IP	30	Saline
Triprolidine	50, 100, 200, 400, or 800 μg	IP	30, 60, or 120	Saline
JNJ-777120	20 mg/kg	SC	30	4.4% DMSO in saline
Resveratrol	10 mg/kg	OG	60	2% DMSO in saline
Formoterol	10, 20, or 40 μg/kg	IV	10, 37, 75, or 150	0.6% DMSO in saline
Theophylline	5 mg/kg	IP	30	Saline
Cromolyn	300 μg	IP	30	Saline
Curcumin	50 mg/kg	OG	60	7.3% DMSO in saline
Kaempferol	50 mg/kg	OG	60	7.3% DMSO in saline
Quercetin	50 mg/kg	OG	60	7.3% DMSO in saline
Indacaterol	2.5, 5, or 10 mg/kg	IV	30	2.3% DMSO in saline
	or 2.5 mg/kg	SC	37, 60, 75, 150, 300, or 600	0.55% DMSO in saline
Doxepin	10 mg/kg	IP	5	Saline
Terbutaline	0.5 or 2 mg/kg	SC	30	Saline
Albuterol	0.078125, 0.15625, 0.312, 0.625, 1.25, 2.5, 5, 10, or 20 mg/kg	IP	30	Saline
Idelalisib	5, 10, 20, 40 or 80 mg/kg	IP	30	30% polyethylene glycol 400/0.5% Tween 80/5% propylene glycol
Ibrutinib	10 or 25 mg/kg	IP	30	1.8% DMSO in saline
Ketotifen fumarate	25 mg/kg	IP	30	25% DMSO in saline
Zileuton	50 mg/kg	OG	24 or 60	23.8% DMSO in saline
Montelukast	6 mg/kg	SC	24 or 60	16.7% DMSO in saline
Ranitidine	100 mg/kg	IP	30	PBS
Epinephrine	2, 10, or 50 μg	SC	5 (postchallenge)	Saline

OG, Oral gavage.

TABLE E2. Effects of individual agents on IgE-mediated anaphylaxis

Target	Inhibitor/agonist*	Effect on FcεRI-mediated induction of hypothermia
HR1	Triprolidine	Considerable dose-related inhibition
	Doxepin†	Considerable inhibition, similar to triprolidine
	Ketofen‡	Less effective inhibition than triprolidine or doxepin
HR2	Ranitidine	No effect
HR4	JNJ-777120	Tendency toward mild inhibition, but not significant
β-adrenergic receptor	Formoterol*	Considerable dose-related inhibition
	Terbutaline*	Considerable dose-related inhibition
	Albuterol*	Considerable dose-related inhibition
	Indacaterol*	Considerable dose-related inhibition
α- and β-adrenergic receptors	Epinephrine*	Direct toxic effect, induces hypothermia
Syk	Fostamatinib§	Considerable dose-related inhibition
BTK	Ibrutinib	Little or no effect
PI3K P110δ	Idelalisib	Little or no effect
Abl/Kit	Imatinib	Little or no effect
Mast cells	Cromolyn sodium	Little or no effect
5-lipoxygenase	Zileuton	Little or no effect
CysLT1	Montelukast	Little or no effect
Leukotriene D4R	REV 5901	Little or no effect
PAFR	ABT-491	Little or no effect
$5\text{-HTR}_{1/2/2a/7}$	Metergoline	Little or no effect
	Ketanserin	
Corticosteroid receptor	Dexamethasone	Little or no effect
Multiple targets and antioxidant effects	Resveratrol (phenol/phytoalexin)	Little or no effect
	Quercetin (flavonoid polyphenol)	Little or no effect
	Kaempferol (antioxidant flavanol)	Little or no effect
	Curcumin (phenol diarylheptanoid)	Little or no effect
Multiple targets	Theophylline	Moderate inhibitory effect

HTR, Hydroxytryptophan receptor; *PAFR*, platelet-activating factor receptor.
*Agonist.
†Inhibits both HR1 and HR2.
‡Inhibits HR1 and 5-HTRs.
§Also suppresses FcεRI desensitization.

TABLE E3. Effects of combinations of agents on IgE-mediated anaphylaxis

Drug combination	Targets	Suppressive effect on FCεRI-mediated hypothermia
Triprolidine + ranitidine	HR1, HR2	Equal to triprolidine alone
Triprolidine + JNJ-777120	HR1, HR4	Tendency to be greater than triprolidine alone, but not significant
Triprolidine + albuterol	HR1, β-AR*	Greater than either drug alone
Triprolidine + indacaterol	HR1, β-AR*	Greater than either drug alone
Triprolidine + terbutaline	HR1, β-AR*	Greater than either drug alone
Triprolidine + fostamatinib	HR1, Syk	Greater than either drug alone
Triprolidine + idelalisib	HR1, PI3K P110δ	Equal to triprolidine alone
Triprolidine + ibrutinib	HR1, BTK	Greater than either drug alone
Triprolidine + imatinib	HR1, Abl/Kit	Equal to triprolidine alone
Albuterol + idelalisib	β-AR,* PI3K P110δ	Equal to albuterol alone
Albuterol + ibrutinib	β-AR,*BTK	Greater than either drug alone
Albuterol + imatinib	β-AR,*Abl/Kit	Equal to albuterol alone
Indacaterol + fostamatinib	β-AR,*Syk	Greater than either drug alone
Triprolidine + indacaterol + fostamatinib	HR1, β-AR,* Syk	Similar to any of the 2 drug combinations
Triprolidine + albuterol + fostamatinib	HR1, β-AR,* Syk	Similar to triprolidine + albuterol
Imatinib + fostamatinib	Abl/Kit, Syk	Greater than either drug alone
Idelalisib + fostamatinib	PI3K P110δ, Syk	Greater than either drug alone
Ibrutinib + fostamatinib	BTK, Syk	Greater than either drug alone

β-AR, β-adrenergic receptor.
*Agonist.