The soluble isoform of human FcεRI is an endogenous inhibitor of IgE-mediated mast cell responses

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Abstract

Background: The soluble isoform of FcεRI, the high affinity IgE receptor (sFcεRI), is a protein of the human IgE network with poorly defined functions.

Objective: Define cellular sources and signals that result in the production of human sFcεRI and study its *in vivo* functions.

Methods: FcεRI-transfected human cell lines (MelJuso), human monocyte-derived dendritic cells (moDCs), and murine bone marrow-derived mast cells (MC) were stimulated by FcεRI crosslinking and release of sFcεRI was analyzed in supernatants (ELISA, Western Blot). Murine LAMP-1 (lysosomal-associated membrane protein 1) degranulation assays and human basophil activation tests (BAT) were used to study IgE-dependent activation. Recombinant sFcεRI

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(rsFcεRI) was used to assess the role of this soluble IgE receptor in murine models of anaphylaxis with WT (wild type) and IgE$^{-/-}$ mice

Results: Antigen-specific crosslinking of IgE-loaded FcεRI on cell lines that express the trimeric or tetrameric receptor isoform induced the production of human sFcεRI. Using MCs and moDCs, we confirmed that IgE/FcεRI activation induces sFcεRI release. We demonstrated that generation of sFcεRI requires Src phosphorylation and endo/lysosomal acidification. In experimental mouse models, sFcεRI diminishes the severity of IgE-mediated anaphylaxis *in vivo*. BATs confirmed that observation, comparable to the humanized anti-IgE monoclonal antibody omalizumab, sFcεRI is an inhibitor of the human innate IgE effector axis, implying that sFcεRI and omalizumab potentially inhibit each other *in vivo*.

Conclusion: sFcεRI is produced after antigen-specific IgE/FcεRI-mediated activation signals and functions as an endogenous inhibitor of IgE-loading to FcεRI and IgE-mediated MC activation. Our results imply, therefore, that sFcεRI contributes to a negative regulatory feedback loop that aims at preventing overshooting responses after IgE-mediated immune activation.

INTRODUCTION

Allergen-specific crosslinking of IgE-loaded FcεRI on mast cells (MCs) and peripheral blood basophils is a central activation event in many types of allergies. Activation of this IgE/FcεRI effector axis is also pivotal to IgE-hypersensitivity reactions which, when elicited by food, range from oral tingling and swelling to life-threatening anaphylaxis (1, 2). For food allergy, no treatments other than allergen avoidance and emergency management with epinephrine, corticosteroids, and antihistamines are currently available to the general public. Therefore, there is a high priority to study the IgE/FcεRI pathway because such research offers opportunities to discover new therapeutic intervention targets for allergy and anaphylaxis.

FcεRI is a multimeric immune recognition receptor of the IgE network and has been studied in great detail due to its crucial role in allergy (3, 4). The tetrameric isoform consists of the IgE-binding alpha chain, the beta chain, and the gamma chain dimer; the latter two subunits serving as signaling proteins (1). Tetrameric FcεRI is constitutively expressed on the surface of immune cells of the innate IgE effector axis, such as MCs and basophils, in rodents and humans. During the acute phase of an allergic response, the cell-bound fraction of antigen-specific IgE pool gets crosslinked and induces an activation cascade that results in the immediate release of preformed intracellular mediators such as histamine. Additionally, the innate cells start to produce inflammatory Th2-type cytokines that perpetuate allergic inflammation (4, 5).

In humans, dendritic cells (DCs) constitutively express a trimeric isoform of FcεRI which has been described to have a regulatory function by contributing to the clearance of serum IgE (6) and by providing a feedback mechanism that restrains allergic tissue inflammation

(7, 8). The trimeric isoform of FcεRI can be induced by virus infections on murine DCs (9) but is not constitutively expressed in wild type (WT) mice. In addition to the two transmembrane FcεRI isoforms, a truncated version of the alpha chain, further referred to as soluble FcεRI (sFcεRI) has been described in human serum (10, 11). Currently, the cellular source of sFcεRI in humans or the signaling requirements for its generation are poorly defined. Previous studies have shown that sFcεRI is released after IgE-crosslinking and this soluble IgE receptor prevents IgE-binding to different FcεRI-expressing cell lines (MelJuso (10) and RBL-2H3), and interferes with PCA (passive cutaneous anaphylaxis) response in rats (12). Several open questions with regards to the biology of sFcεRI and its role within the IgE-network (3) remain to be addressed. Specifically, the signaling requirements for sFcεRI production have not been defined and, furthermore, it remains to be tested how sFcεRI affects IgE-mediated anaphylaxis *in vivo*.

Omalizumab, a recombinant humanized anti-IgE monoclonal antibody, has been established as a treatment for uncontrolled allergic asthma and later for CSU, Chronic Spontaneous Urticaria (13). Omalizumab binds to the Fc portion of IgE and has been shown to interact with free IgE that circulates in serum (14). Initially, this type of anti-IgE therapy leads to an increase in IgE levels followed by a decrease of IgE and FcεRI expression on DCs, MCs, and basophils (15–19). Since omalizumab and sFcεRI can both interact with IgE in human serum, it is important to address if the anti-IgE antibody and the soluble IgE Fc receptor potentially overlap in their function.

Here we show that crosslinking of tetrameric and trimeric surface FcεRI induces the production of the soluble receptor isoform in cell line models and primary human cells. In addition to IgE-mediated receptor activation, generation of sFcεRI depends on Src signals and requires endo/lysosomal acidification. Comparable to omalizumab, sFcεRI inhibits FcεRI-mediated basophil activation, and experimental models of murine allergy showed that sFcεRI ameliorate antigen-induced anaphylaxis *in vivo*.

MATERIALS & METHODS

sFcεRI ELISA

Levels of soluble FcεRI (sFcεRI) from supernatants were measured by a commercial ELISA according to manufacturer's protocol (BMS2101, Thermo Fisher Scientific,Waltham, MA, USA).

Cells and culture conditions

Human melanoma-derived cell line MelJuso were transfected with FcεRIαγ, FcεRIαβγ, or empty vector (MelJuso-αγ, MelJuso-αβγ, and MelJusoØØ, respectively), cultured as previously described (10, 20), and used to study production of sFcεRI and its function *in vitro*.

For the generation of monocyte-derived dendritic cells (moDCs), monocyte isolation from heparinized blood was performed with CD14 microbeads by MACS separation according to manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were cultured in RPMI-medium supplemented with 10% FBS (Gibco), 10000 U/mL Pen-Strep

(Gibco), 2 mM L-Glutamine and 0.1 mM non-essential amino acids, with the addition of IL-4 (Interleukin-4) and GM-CSF (granulocyte-macrophage colony-stimulating factor) (1000U/mL, PeproTech, Rocky Hill, USA).

For the generation of murine bone marrow-derived mast cells (MCs), femurs of hFcεRIα+/mFcεRIα−/− and mFcεRIα−/− mice were flushed an progenitor cells were cultured as described (21) in RPMI-medium supplemented with 10% FBS (Gibco, Thermo Fisher Scientific,Waltham, MA, USA), 10000 U/mL Pen-Strep (Gibco, Thermo Fisher Scientific,Waltham, MA, USA), 2 mM L-Glutamine, 50 μM β-mercaptoethanol (Gibco, Thermo Fisher Scientific,Waltham, MA, USA), 10 ng/mL recombinant murine IL-3 (Interleukin-3) and SCF (stem cell factor) (PeproTech, Rocky Hill, USA).

Flow cytometry analysis

Cells were harvested, washed and centrifuged at 330 g at 4ºC with staining-buffer (DPBS 1X supplemented with 2% FBS and 0.1% NaN_3). Cells were stained for 15 minutes at 4°C with NP-PE (4-Hydroxy-3-nitrophenylacetyl hapten conjugated to PE (Phycoerythrin) protein through lysine by amide bonds) (Biosearch Technologies, Petaluma, CA, USA) for cIgE (chimeric humanized anti-NP IgE, MCA333S clone JW8/1 BioRad, Serotec) detection, CRA-1-FITC or -APC (mouse IgG2b,K anti-human FcεRIα, clone AER-37) and MAR-1-APC (hamster IgG anti-murine FcεRIα) (BioLegend, San Diego, CA, USA) for human and murine FcεRIα detection respectively, IgE-PE ((BioLegend, San Diego, CA, USA) for murine IgE detection, CD49b-Pacific Blue™ (BioLegend, San Diego, CA, USA) for murine basophil characterization, CD1a-APC (mouse IgG1, K anti-human CD1a, clone HI149) (BioLegend, San Diego, CA, USA) for dendritic cell characterization, c-Kit-FITC (BioLegend, San Diego, CA, USA) for mast cell characterization, CD107a-PE/Cy7 (BioLegend, San Diego, CA, USA), and Fixable Viability Dye eFlour™ 660 (eBioscience, Thermo Fisher Scientific, Waltham, MA, USA) for LAMP-1 assays. Basophils (50 μL whole blood) were IgE-stripped with lactic acid (pH=3.9) for 5 minutes and loaded with cIgE in presence or absence of recombinant sFcεRI (rsFcεRI) or omalizumab for 1 hour at 37ºC. Basophil stimulation (NP-OVA (4-Hydroxy-3-nitrophenylacetyl ovalbumin), 2.85 μM) and staining with CD63-FITC and CCR3-PE were performed according to manufacturer's protocol (Flow CAST, Bühlmann, Schönenbuch, Switzerland). Data was acquired on a FACS-Canto II flow cytometer and analysis was performed by FlowJo v10.

FcεRI crosslinking assays

MelJuso cells ($5×10^5$ cells), moDCs ($1.7–2.5×10^6$ cells), and MCs ($5×10^5$ cells) were loaded overnight with cIgE (from 0.1to10 μg/mL) and stimulated with NP-OVA or -BSA (4-Hydroxy-3-nitrophenylacetyl bovine serum albumin) (from 0.1 to 100 μg/mL) for 2–24 hours. MelJuso cells were incubated with TAPI-2 (18–72 μM) for 30 minutes prior to NP-OVA stimulation. Omalizumab (0.27–0.8 μM) and sFcεRI (25–500 nM) were incubated with cIgE for 30 minutes prior to loading on MelJuso and basophils. Supernatants from cell cultures were collected between 2–24 hours after FcεRI crosslinking and analyzed by ELISA or Western Blot.

LAMP-1 degranulation assays

Mature murine MCs (1×10^5 cells per condition), defined as at least 8 weeks old cells with a purity of greater than 90% assessed by cell surface coexpression of mast cell markers c-Kit and FcεRIα by flow cytometry, were loaded overnight with cIgE (500 ng/mL). The next day, cells were washed twice with medium, plated in a 96-well tissue culture plate (10^5 cells in 100 μL per well), and challenged for 10 minutes with a cocktail containing NP-BSA (100 ng/mL) as antigen for IgE-specific receptor activation, an anti–mouse LAMP-1 antibody, and a viability dye (22). Following 2 washes with cold staining-buffer, cells were acquired on a FACS Canto II flow cytometer (BD Bioscience) and further analyzed using FlowJo v10.

Murine anaphylaxis and testing of inhibitory features of sFcεRI *in vivo*

Age- and sex-matched BALB/c wild type (WT) and IgE deficient (IgE$^{-/-}$) mice (23) were allocated to experimental groups. Mice were passively (n=3, 3–6 mice per group; total of 19 mice) sensitized with IgE-anti-DNP (dinitrophenyl) (5 μg) in presence or absence of human rsFcεRI (3 μg) by retro-orbital (r.o.) injections. Mice sensitized with heat-denatured IgE-anti-DNP (3 hours at 65°C) were used as a control. Active (n=2, 3–4 mice per group; total of 24 mice) sensitization was performed by intra-peritoneal (i.p.) injections of OVA/ Alum (ovalbumin/aluminum hydroxide and magnesium hydroxide) (100 μg) once a week for three weeks. Experimental groups received additionally rsFcεRI (3 μg) by i.p. injection 24 hours prior challenge. Mice were challenged with DNP-OVA (100–200 μg) or OVA (500 μg) by i.p. injections for passive and active sensitization models respectively. Mice challenged with PBS were used as a control. Drop of core body temperature was used as a readout for systemic anaphylaxis and was recorded up to one hour after challenge (24, 25).

Inhibition of IgE/FcεRI signaling

Murine MCs (1×10^5 and 5×10^5 cells per condition) were loaded overnight with cIgE (500 ng/mL) followed by 30 minutes incubation with 5–50 μM PP2 (Sigma-Aldrich, Merck, St. Louis, MO, USA) or 20–50 μM chloroquine (Sigma-Aldrich, Merck, St. Louis, MO, USA). NP-BSA (100 ng/mL) was added for 10 minutes and LAMP-1 degranulation assays were performed. IgG anti-human FcεRIα antibodies 19–1 and 15–1 (26) were used (1 and 10 ng/mL) in absence of cIgE and NP-BSA for 2 hours. Same stimulation conditions were performed in parallel and supernatant was collected after 2–24 hours for ELISA analysis.

RESULTS

Antigen-mediated IgE-crosslinking of tetrameric and trimeric FcεRI results in production of sFcεRI

To test if sFcεRI was released by the tetrameric and/or trimeric isoform of FcεRI, we first used cell lines (MelJuso-αγ and MelJuso-αβγ (10)). MelJuso cell lines that stably express trimeric or tetrameric FcεRI have been published as suitable model to study IgE binding to different FcεRI isoforms and receptor activation via antigen-specific IgE-mediated crosslinking (10, 20). Cell lines were loaded with hapten NP-specific (4-Hydroxy-3-nitrophenylacetyl) chimeric IgE (cIgE) overnight. After removal of excess cIgE, cells were treated with haptenized bovine serum albumin (NP-BSA) or ovalbumin (NP-

OVA) to activate IgE-loaded FcεRI. Supernatants from cell cultures were harvested and analyzed by Western Blot (Figure 1A). Supernatants from MelJusoØØ cells served as negative controls as they neither express nor release sFcεRI. Low sFcεRI levels were detected in supernatants from unloaded and cIgE-loaded MelJuso-αγ and MelJuso-αβγ cells and were significantly increased after crosslinking, which demonstrates that activation of both human receptor isoforms induces production of sFcεRI.

To confirm out findings with human primary cells, we generated monocyte-derived DCs (moDCs) defined as CD1a$^+$/FcεRIα$^+$ cells by FACS analysis (Figure 1B). moDCs were loaded with cIgE and activated as described for MelJuso cells earlier in the manuscript. After receptor crosslinking, sFcεRI was found in supernatants from moDC cultures of four individual donors. Low amounts of sFcεRI were released from moDC from 1 of 4 donors in the absence of IgE-mediated activation. IgE-mediated cell activation induced or significantly increased production of sFcεRI ($p<0.05$) in all cultures (Figure 1C).

Production of sFcεRI requires Src kinase activation and endo/lysosomal acidification

In order to understand the process of sFcεRI release, we used MelJuso-αγ cells in presence of a potent inhibitor of matrix metalloproteinases (MMP); a class of proteases that has been described as being responsible for the shedding of several surface proteins (27–29). Cells were loaded with cIgE overnight and incubated with the inhibitor TAPI-2 for 30 minutes before receptor activation with NP-OVA (Supplementary Figure 1A). Supernatants were harvested after 2 hours of activation and analyzed by ELISA. TAPI-2 treatment resulted in a non-significant reduction of sFcεRI production (Supplementary Figure 1B). Our results indicate that MMPs might be partially involved but not solely responsible for the release of sFcεRI.

To further investigate signaling requirements for the production of sFcεRI, we generated bone marrow-derived MCs from mice that were humanized for the expression of the human alpha chain of FcεRI (21, 24, 30). These animals were generated on the murine FcεRIα–/– background and, therefore, express FcεRI on MCs as a chimeric tetrameric receptor of the human alpha chain with the murine signal transducing receptor units. MCs, according to literature (31) and defined as c-Kit$^+$/FcεRIα$^+$, were loaded with cIgE on the chimeric FcεRI and the receptor internalized after NP-BSA stimulation (Supplementary Figure 2).

LAMP-1 (lysosomal-associated membrane protein 1) degranulation assays were used as readout for IgE/FcεRI-mediated MC activation (Figure 2A). In line with previous studies, FcεRI internalized, dependent on the used crosslinking conditions, with an average degranulation rate of 40% (Figure 2B). Comparable to human moDCs, human sFcεRI was detected in supernatants of murine humanized MCs 2 hours after antigen-specific crosslinking (Figure 2C). Release of sFcεRI in the supernatant was also correlated with β-hexosaminidase release (data not shown), another common marker of MCs activation (32). To further assess the production of sFcεRI after receptor activation, we crosslinked with the human alpha-chain specific antibodies, 19–1 and 15–1, that target two different epitopes of the alpha-chain of the FcεRI which are both located close to the IgE binding site and induce crosslinking in an IgE-independent manner (33). Antibody-mediated crosslinking of

the receptor did not provide a signal that increased the production of sFcεRI, at least not at the concentration tested in our assay (Figure 2C).

The signaling cascade of tetrameric FcεRI is far better understood than that of the trimeric isoform. Therefore we further investigated the signaling requirements for sFcεRI production with MCs from FcεRIα-humanized mice. IgE-mediated activation of MCs triggers a series of FcεRI signaling events starting with phosphorylation of ITAM domains by Src kinases, specifically Lyn and Syk, followed by receptor internalization and degradation by the endo/lysosomal compartment (34, 35). Consequently, we tested the effects of the Src inhibitor PP2 and the endo/lysosomal acidification inhibitor chloroquine on the production of sFcεRI by MCs. By blocking Src kinases (36, 37), the ITAM-dependent signaling cascade after receptor crosslinking will be stopped. When receptor activation-crosslinking occurred in presence of PP2, degranulation and sFcεRI production (24 hours after activation) were significantly inhibited (Figure 2). A significant inhibition of sFcεRI production was also observed in the presence of chloroquine, an acidification inhibitor, which impairs receptor internalization and degradation (Figure 2D–E). When both inhibitors were combined, MC degranulation and sFcεRI production was completely abrogated.

Human sFcεRI blocks loading of IgE on cell surface FcεRI and inhibits basophil activation

The ability of sFcεRI to interfere with IgE-loading on the receptor was first tested on MelJuso cells. When MelJuso-$\alpha\gamma$ and MelJuso–$\alpha\beta\gamma$ cells were loaded with cIgE in the presence of either endogenously produced sFcεRI or recombinant sFcεRI (rsFcεRI), the binding of IgE to the cell surface was impaired in a dose dependent manner (Supplementary Figure 3). The effect of endogenously produced and recombinant sFcεRI on loading of surface receptor was comparable. MelJusoØØ cells were used as negative control and showed no binding of IgE.

Since sFcεRI seemed to have a strong blocking effect on IgE-binding to the surface receptor, we compared its effect to widely studied humanized monoclonal antibody omalizumab, which binds to IgE-Fc and blocks the FcεRI-binding site (15, 17). Using the same setting, the inhibitory effect of rsFcεRI was comparable to omalizumab (Figure 3A and Supplementary Figure 4).

To assess functional consequences of the inhibition of IgE-binding, we performed modified Basophil Activation Tests (BAT). Surface IgE was stripped from human basophils from healthy donors. Thereafter, basophils were re-loaded with cIgE in the presence or absence of rsFcεRI or omalizumab and stimulated with NP-OVA. CD63 expression was used as readout for basophil activation (Figure 3B–C). Compared to unstimulated basophils (Supplementary Figure 5), the expression levels of CD63 increased after activation of cIgE-loaded basophils (Figure 3C). Basophil activation was diminished by rsFcεRI and omalizumab (Figure 3C). A significant inhibition was achieved by sFcεRI (0.5μM), and the effect was comparable to omalizumab (0.8μM) despite the lower concentration. Two positive controls, FcεRI-dependent and -independent (C1 and C2 respectively) were used to ensure viability of basophils.

sFcɛRI is an *in vivo* modulator of IgE-mediated immune responses

Our next aim was to investigate how the presence of sFcɛRI in serum would affect IgE-mediated anaphylaxis. It is important to note here that murine IgE can bind to human FcɛRIα and murine FcɛRIα with comparable binding kinetics (30, 38). WT BALB/c mice were passively sensitized by retro-orbital injections using murine IgE-anti-DNP (dinitrophenyl) in the presence or absence of human rsFcɛRI 24 hours before a challenge with DNP-OVA (Figure 4A and Supplementary Figure 6A for the characterization of rsFcɛRI). Challenge-induced drop in core body temperature was observed in IgE-sensitized mice. Importantly, animals that were sensitized in the presence of rsFcɛRI responded with a significantly diminished drop in temperature (Figure 4B). Mice that were injected with IgE and rsFcɛRI, but not challenged and animals that received only rsFcɛRI were used as specificity controls and did not show alterations of body temperature (Figure 4C). An experimental condition in which we used heat-denatured IgE (dIgE) for passive sensitization confirmed that our experimental settings specifically monitor IgE-mediated anaphylaxis (Figure 4C).

Symptoms of murine anaphylaxis observed in passive sensitization experiments are commonly rather moderate (39). As a complementary approach, we therefore addressed whether rsFcɛRI would be able to improve symptoms of anaphylaxis after systemic immunization. WT BALB/c mice were sensitized with OVA/Alum (ovalbumin/aluminum hydroxide and magnesium hydroxide) injections once a week for three weeks. The experimental group received a single rsFcɛRI injection 24 hours before antigen-challenge while the control group was injected with PBS alone (Figure 4D). Animals responded to antigen-challenge with a pronounced temperature drop from which they started to recover after 40 minutes (Figure 4E and Supplementary Figure 6). Temperature of mice that were injected with rsFcɛRI 24 hours before challenge dropped significantly less and animals started to recover as early as 20 minutes after challenge.

To assess if sFcɛRI specifically impairs IgE-mediated anaphylaxis, we performed side-by-side experiments in WT and IgE-deficient (IgE$^{-/-}$) animals. Both strains were sensitized and challenged using the experimental outline detailed in Figure 4D. Using a different aliquot of rsFcɛRI, we confirmed that the temperature of WT mice that received a single dose of rsFcɛRI 24 hours before challenge suffered from less pronounced anaphylaxis than untreated WT animals (Figure 5A and C). In IgE$^{-/-}$ animals, in which other pathways than IgE-mediated FcɛRI crosslinking on MCs are responsible for the induction of anaphylaxis (40, 41), the protective effect of rsFcɛRI was not observed (Figure 5B and C). When measuring IgE on peripheral blood basophils, we found that basophils from WT mice that were treated with rsFcɛRI expressed lower levels of surface IgE than controls (Figure 5D and E). As expected, basophils from IgE$^{-/-}$ mice had no surface IgE (Figure 5E). In summary this set of experiments confirms that sFcɛRI specifically affects anaphylaxis induced by the IgE/FcɛRI-mediated activation of the innate effector axis of allergy (7, 34).

DISCUSSION

Here, we demonstrated that sFcɛRI release occurs for the tetrameric isoform when expressed on MelJuso cells and from murine humanized MCs. Most importantly, this

study describes sFcεRI release from human moDCs for the first time. We further delineate the requirements for sFcεRI release, such as antigen-specific receptor crosslinking, phosphorylation, internalization, and intracellular endo/lysosomal acidification. We cannot completely exclude the effect of proteinase-induced shedding, which might also contribute in the release of sFcεRI found in presence of Src kinase or endo/lysosomal acidification inhibitors in MCs. Furthermore, our data indicate that sFcεRI may prevent binding of IgE to FcεRI and thereby, impairs IgE-mediated effector cell activation. This effect was further assessed showing amelioration of anaphylaxis. Our findings, together with the similarities in effect between sFcεRI and omalizumab, provide evidence that sFcεRI is an endogenous modulator of IgE-mediated responses and the induction of its production might be a potential strategy to modulate allergic responses.

It has been previously described that sFcεRI can be found in serum, almost uniquely bound to IgE (42). Our results suggest that both DCs and MCs are potential sources of sFcεRI in serum. However, we cannot exclude that other cell types that express either trimeric or tetrameric FcεRI, such as basophils, eosinophils, macrophages, or Langerhans cells, additionally contribute to the serum levels of sFcεRI. Moreover, FcεRI expression can be induced on neutrophils (43) in allergic asthma and murine DCs after viral infection (9), which might also have an effect on sFcεRI in these particular conditions.

It has been previously shown that sFcεRI can prevent IgE-binding on MelJuso-$\alpha\gamma$ (10) and RBL-2H3 (12) cells. Here we have shown that, *in vitro*, sFcεRI binds to IgE leading to the formation of sFcεRI/IgE complexes, thus lowering the capacity of free IgE to bind to and subsequently activate cell surface FcεRI on MelJuso-$\alpha\beta\gamma$ cells and human basophils. We propose the process of sFcεRI release to be a protective mechanism against subsequent allergen-mediated FcεRI/IgE crosslinking and sustained cellular activation. This hypothesis is supported by *in vivo* experiments in mouse models of anaphylaxis in the current publication. When rsFcεRI was injected at the time of sensitization, it was sufficient to protect from anaphylaxis. If rsFcεRI was injected in a systemic sensitization model, it resulted in a diminished severity and improved recovery of IgE-mediated anaphylaxis, as well as a decrease in bound IgE to basophils. An additional argument for the specificity of the inhibitor activity of sFcεRI for the IgE/FcεRI-mediated pathway of anaphylaxis is derived from the observation that no amelioration of IgE-independent anaphylaxis (40, 41) was observed in IgE$^{-/-}$ animals. The strong immune response triggered in the active sensitization model, compared to the passive sensitization model, might explain why we still observe a temperature drop in presence of rsFcεRI, which suggests that the IgE:rsFcεRI ratio may important. These results imply that sFcεRI is an *in vivo* modulator of IgE-mediated responses, and support our proposed mechanism of a negative regulatory feedback loop. It is tempting to speculate that sFcεRI could be used as an adjuvant medication during oral immune therapy to prevent immediate responses during therapeutic antigen exposure.

Omalizumab, the humanized anti-IgE antibody, has proven to be a successful treatment for diverse IgE-mediated diseases such as severe allergic asthma, rhinitis or Chronic Spontaneous Urticaria (19, 44–49, 16, 50, 51). In addition, omalizumab has occasionally been used for the treatment of food allergy in combination with food-specific immunotherapy and successfully reduced IgE-mediated side effects (19, 52, 53).

Furthermore, studies based on IgE-trapping molecules have shown potential to modulate IgE-mediated responses, such as anti-IgE Designed Ankyrin Repeat proteins (DARPins), which show inhibition of basophil activation and release of proinflammatory mediators (54), and PepE that prevents passive systemic anaphylaxis (25).

Based on the similarities between omalizumab and sFcεRI, and the emerging research on IgE-trapping strategies, we compared both molecules, and both could effectively inhibit IgE-binding to the receptor as well as IgE-mediated basophil activation. This effect was observed even at low concentrations of sFcεRI as compared to omalizumab, indicating a high affinity and inhibitory capacity of sFcεRI. Our data suggest that both molecules have a similar functional effect as they compete for the same binding domain on IgE, indicating that monitoring of sFcεRI prior to omalizumab therapy might be necessary to identify patients in which the serum IgE binding site is already occupied by an endogenous inhibitor. However, further research on circulating sFcεRI/IgE complexes and human data are needed to fully understand this mechanism.

In conclusion, although the possible implication of sFcεRI as an interference molecule in the IgE-network has been considered and partially investigated, we provide strong evidence that human sFcεRI is released from moDCs and MCs, and it might be part of a negative regulatory feedback loop to prevent detrimental IgE-mediated immune activation. The development of strategies to boost endogenous sFcεRI levels might be a potential therapeutic strategy for prevention and treatment of IgE-mediated allergies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

Alum	aluminum hydroxide and magnesium hydroxide
BAT	Basophil Activation Test
cIgE	chimeric humanized anti-NP immunoglobulin E
CSU	Chronic Spontaneous Urticaria
DARPins	anti-IgE Designed Ankyrin Repeat proteins
DC	dendritic cell

DNP	dinitrophenyl
FcεRI	Fc epsilon Receptor I, high affinity IgE Fc receptor
GM-CSF	granulocyte-macrophage colony-stimulating factor
fMLP	N-formyl-methionyl-leucyl-phenylalanine
hFcεRI	human Fc epsilon Receptor I, high affinity IgE Fc receptor
IgE	Immunoglobulin E
IgE$^{-/-}$	Immunoglobulin E deficiency
IL-3	Interleukin-3
IL-4	Interleukin-4
i.p.	intra-peritoneal
LAMP-1	lysosomal-associated membrane protein 1
MC	mast cell
mFcεRI	murine Fc epsilon Receptor I, high affinity IgE Fc receptor
MMP	matrix metalloproteinase
moDC	monocyte-derived dendritic cell
NP-BSA	4-Hydroxy-3-nitrophenylacetyl bovine serum albumin
NP-OVA	4-Hydroxy-3-nitrophenylacetyl ovalbumin
OVA	ovalbumin
PCA	passive cutaneous anaphylaxis
r.o.	retro-orbital
rsFcεRI	recombinant human sFcεRI
SCF	stem cell factor
sFcεRI	soluble isoform of FcεRI
SPT	skin prick test
WT	wild type

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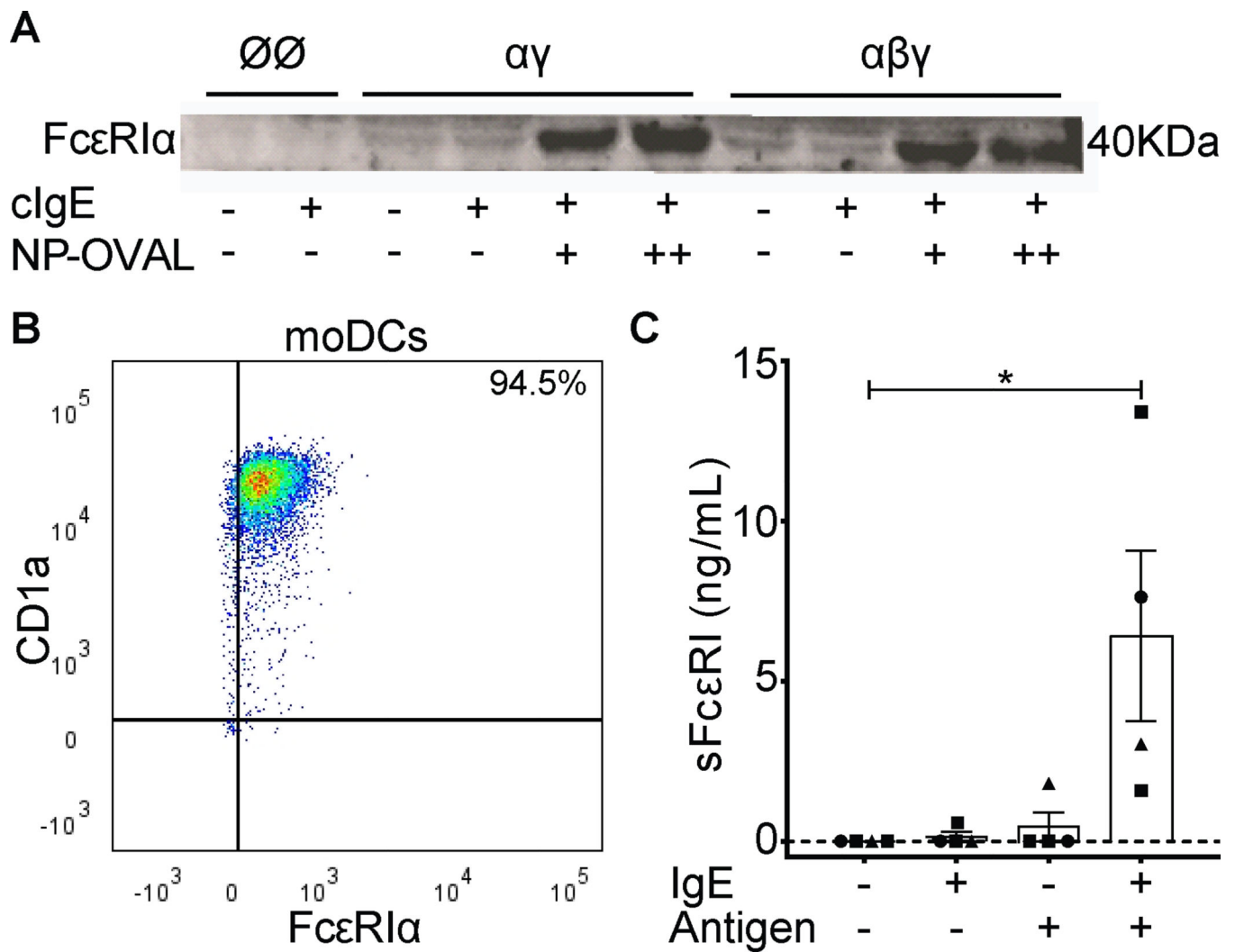
Figure 1. FcɛRI-crosslinking on moDCs induces sFcɛRI production.
Panel **A** shows detection of sFcɛRI by Western Blot analysis from MelJusoØØ/-αγ/-αβγ cell cultures. Panel **B** shows a representative dot plot of mature monocyte-derived DCs. Panel **C** shows detection sFcɛRI by ELISA analysis from monocyte-derived DCs given by mean ± SEM. cIgE (5–10 μg/mL) and NP-OVA (50–100 μg/mL). Individual points represent each donor where squares are males, circles females and triangle unknown. Kruskal-Wallis test plus Dunn's multiple correction was performed where *p<0.05.
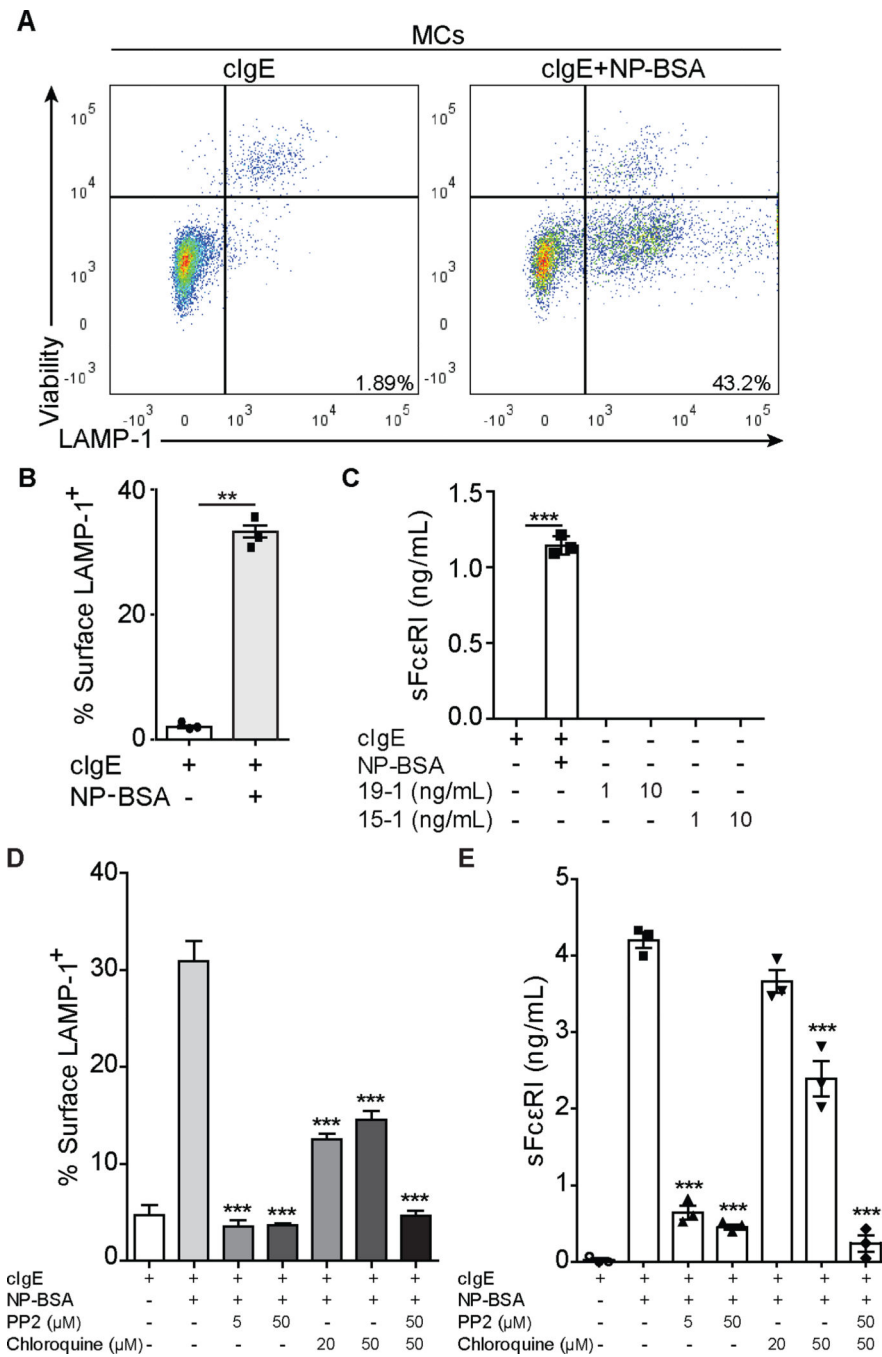
Figure 2. Src kinase activation and endo/lysosomal acidification are required for sFcεRI production.
Detection of surface LAMP-1 expression on bone marrow MCs by flow cytometry (A, B, D) and sFcεRI in supernatants by ELISA (C, E). Panel **A** shows representative dot plots of MCs from LAMP-1 degranulation assays. Panels **B** and **D** show the quantification of percentage of surface LAMP-1+ cells. Panels **C** and **E** show total sFcεRI levels from supernatant of MCs cultures. MCs were loaded overnight with cIgE (500 ng/mL) and added NP-BSA (100 ng/mL) for 10 minutes for LAMP-1 degranulation assays. PP2 and chloroquine were

added for 30 min prior to NP-BSA. In parallel, MCs were loaded overnight with cIgE and added NP-BSA, 19–1, or 15–1 for 2 (19–1 and 15–1) or 24 hours before supernatants were harvested. Graphs show mean ± SEM where paired t-test (B) or 1way ANOVA tests plus Tukey's multiple correction (C-E) was performed; where **$p<0.01$ and ***$p<0.001$ compared to cells incubated with cIgE and NP-BSA.
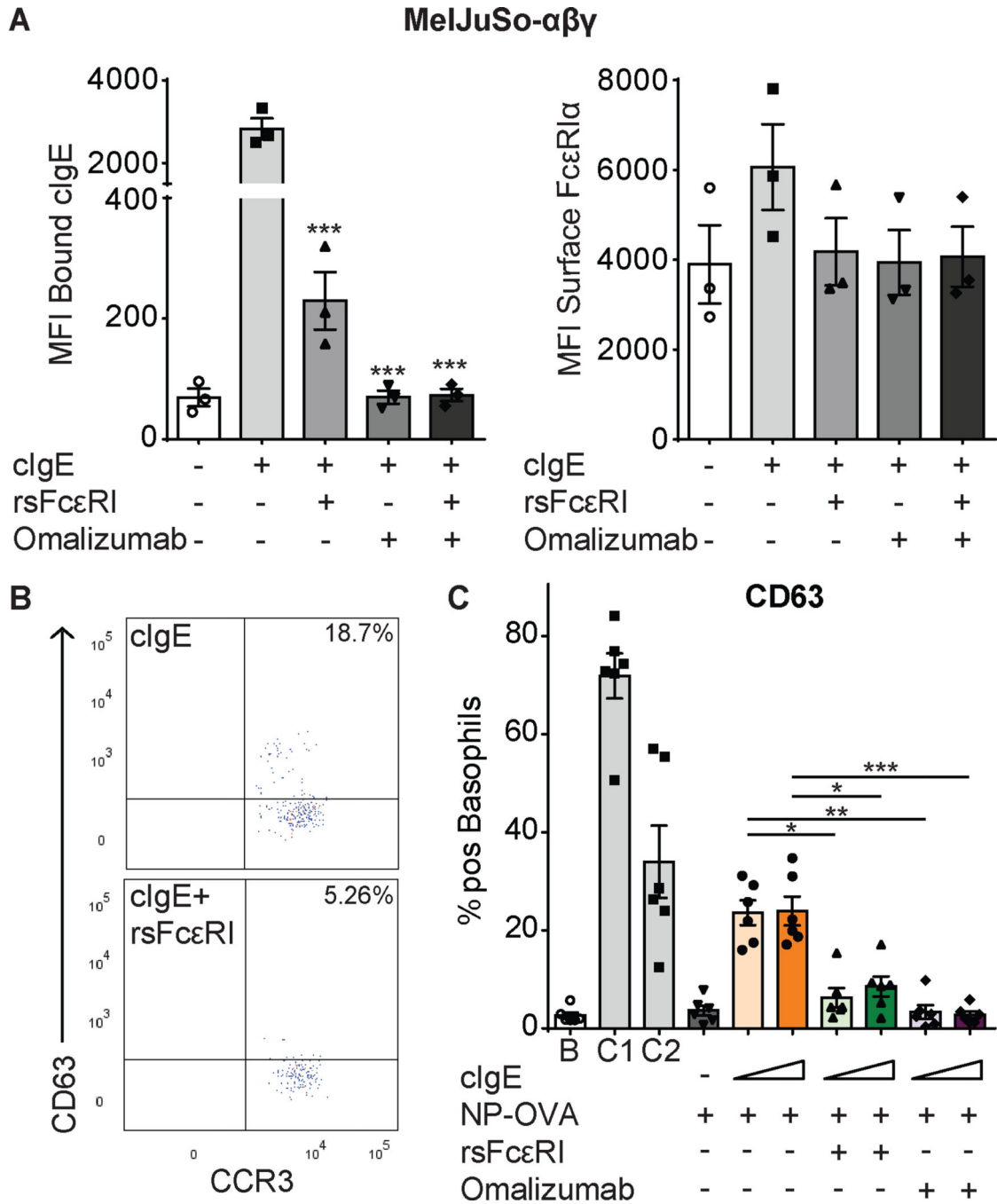
Figure 3. sFcεRI blocks cell surface cIgE binding and basophil activation.
Detection of bound cIgE (A, left) and FcεRIα (A, right) on MelJuso-αβγ (**A**) cells by flow cytometry (sFcεRI (25 nM) and omalizumab (0.27 μM)). Graphs show mean ± SEM. Individual points represent means of independent experiments (n=3). Detection of surface CD63 and CCR3 on basophils by flow cytometry. Panel **B** shows representative dot plots for basophils loaded with cIgE in presence or absence of rsFcεRI. Panel **C** shows the percentage of positive CD63 basophils stimulated with NP-OVA (2.85 μM) and sFcεRI (0.52 μM) or omalizumab (0.8 μM). Graphs show mean ± SEM. Individual points represent each

donor (n=6). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05, **p<0.01, and ***p<0.001, compared to cells loaded with cIgE. B: background; C1: anti-FcεRI mAb; C2: fMLP.
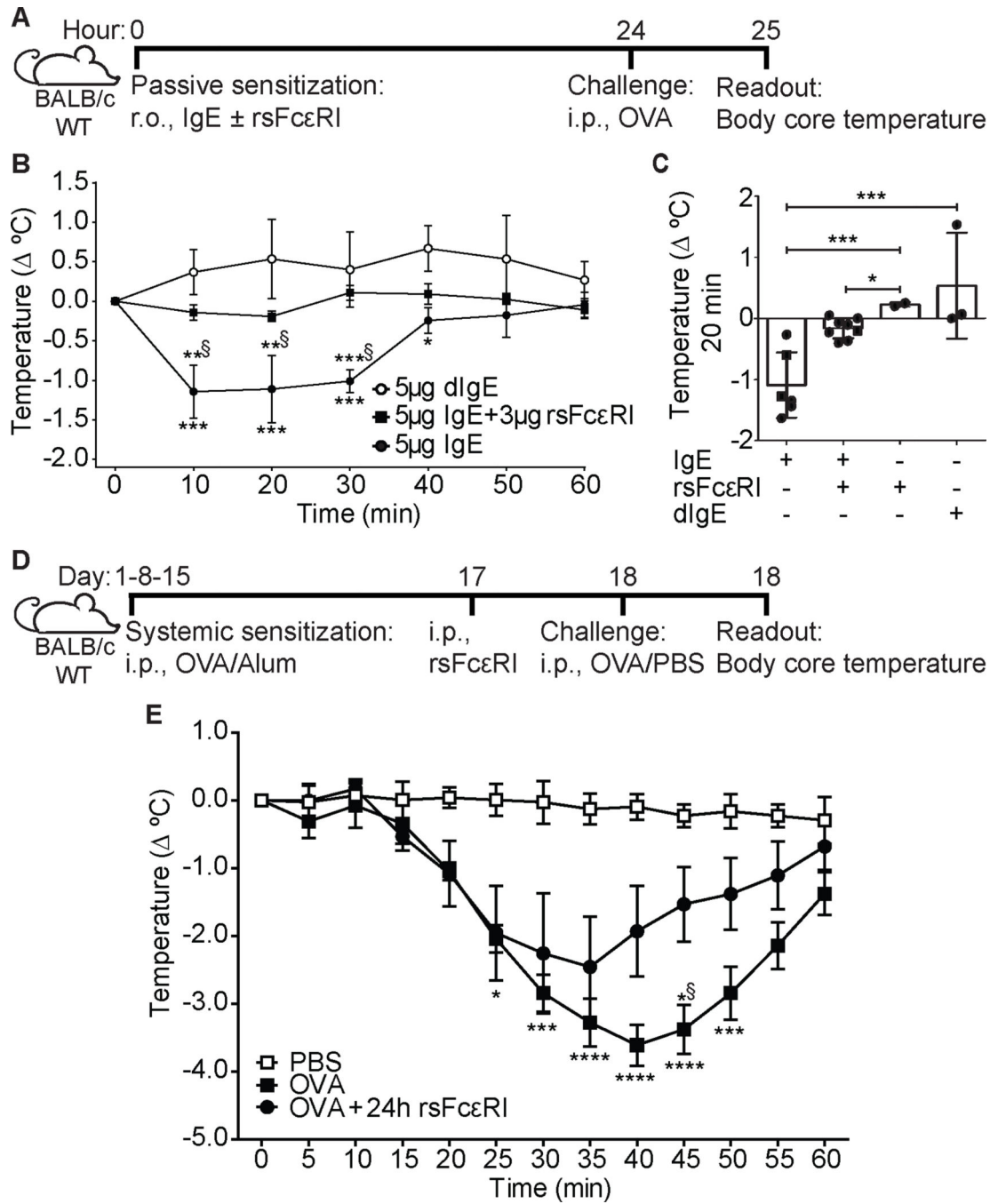
Figure 4. sFcεRI prevents systemic sensitization and improves recovery.

Analysis of core body temperature from passively (A-C) and systemically (D, E) sensitized mice. Panel **A** shows the passive sensitization and challenge strategy. Panel **B** shows the time course of core body temperature drop in sensitized mice that were challenged in the absence or presence of rsFcεRI. Representative experiment, average temperature of 3–6 mice per time point. Panel **C** shows the composite graph of temperature drops at the 20 min time point following DNP-OVA challenge, from three independent experiments (n= 3, total of 19 mice). Panel **D** shows the Th2-type sensitization and challenge strategy. Panel **E** shows the

time course of body temperature drop in sensitized mice that were challenged with OVA in absence or presence of rsFcεRI 24 hours before challenge. rsFcεRI (3 μg), IgE-anti-DNP (5 μg), OVA/Alum (100 μg), and OVA (100–500 μg). Graphs show mean ± SEM with n=3–6/group where 1way ANOVA test and Tukey's multiple correction (C), or 2way ANOVA tests and Tukey's (B) or Bonferroni's (E) multiple correction were performed; *p<0.05; **p<0.01; ***p<0.001, and ****p<0.0001. * represents statistics between IgE and dIgE (B), and PBS and OVA (E) groups, *§ represents statistics between IgE-anti-DNP and IgE-anti-DNP + rsFcεRI (B), and OVA and OVA+24h rsFcεRI (E) groups.
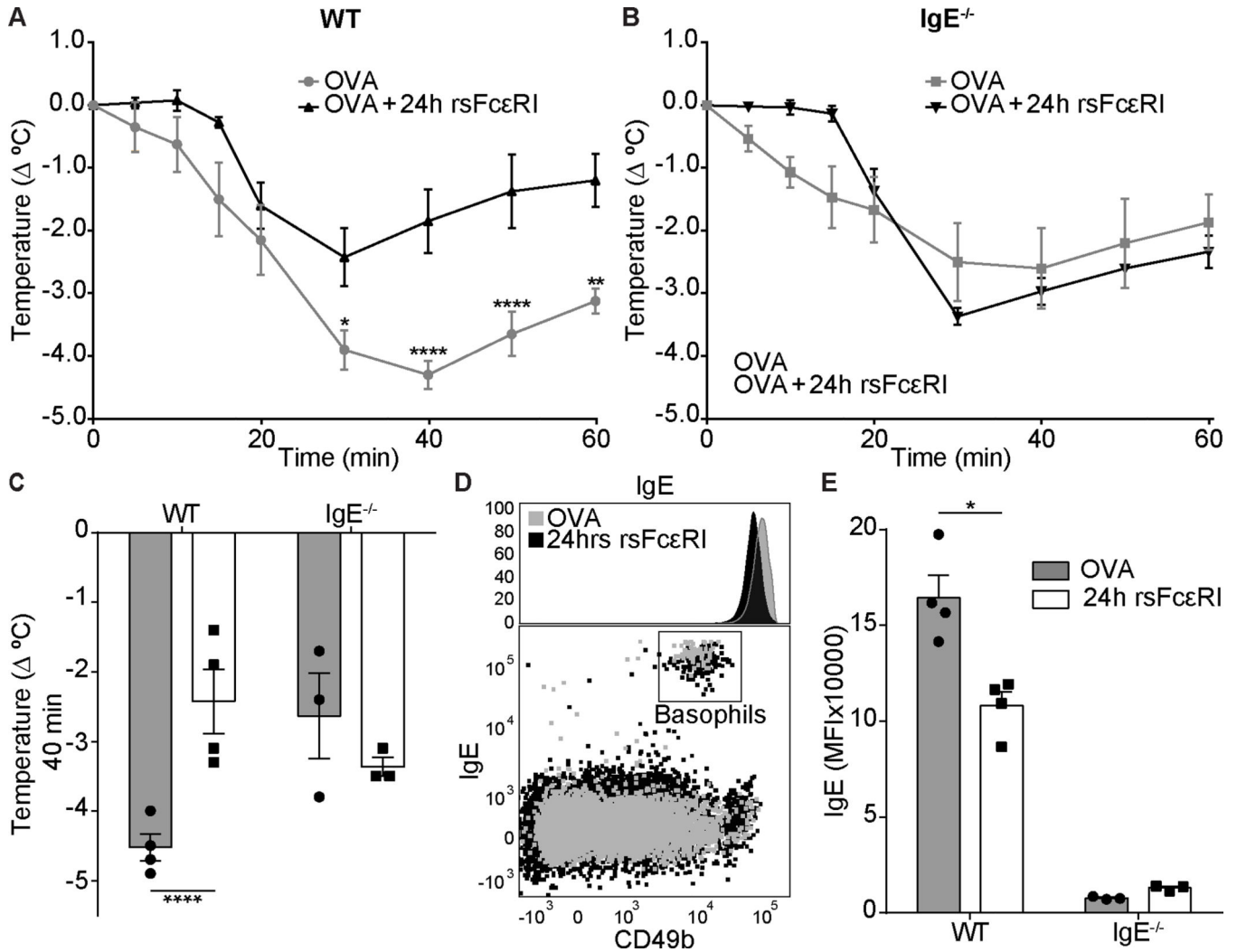
Figure 5. Recombinant sFcεRI prevents anaphylaxis and reduces FcεRI bound IgE on the surface of basophils.

Analysis of rectal temperature from systemically sensitized mice (A-C). Panels **A** and **B** compare the effect of rsFcεRI treatment in a time course of core temperature drop in WT (A) or IgE$^{-/-}$ (B) mice. Panel **C** shows the maximum temperature drop of WT and IgE$^{-/-}$ mice 40 min after OVA challenge. Panel **D** shows a representative histogram and a dot plot of peripheral blood basophils from WT mice with and without rsFcεRI treatment. Panel **E** shows the MFI of surface bound IgE on basophils with and without rsFcεRI treatment. rsFcεRI (3 μg), OVA/Alum (100 μg), and OVA (500 μg). Graphs show mean ± SEM with n=3–4/group where 2way ANOVA tests and Tukey's multiple correction were performed; *p<0.05 and ****p<0.0001.