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METHODS

Reagents

For *in vitro* experiments, chimeric humanized anti-NIP IgE (cIgE) (MCA333S clone JW8/1 Serotec BioRad, Hercules, CA, USA) and its hapten-specific antigen NP-OVA/BSA (N-50051 and N-5050L, Biosearch Technologies, Petaluma, CA, USA) were used at different concentrations (0.1-40 µg/mL and 1-10000 ng/mL respectively). Omalizumab solutions (Novartis, Basel, Switzerland) were used in different dilutions in Dulbecco's phosphate-buffered saline (DPBS) (from 40 µg/mL to 1 ng/mL). TAPI-2 solutions (Santa Cruz Biotechnologies, Dallas, TX, USA) were used at different concentrations (18 and 72 µM). Endogenously produced soluble FcεRI (sFcεRI) was purified from MelJuso cell supernatants, analyzed by ELISA and Western Blot, and used at different dilutions (4-10 ng/mL). Recombinant human sFcεRI (rsFcεRI) was either purchased (MyBioSource, San Diego, CA, USA) or provided by the laboratory of Theodore Jardetzky, PhD (Department of Structural Biology in the School of Medicine at Stanford University) for *in vivo* experiments previously described (1).

Patient information

Allergic patients or adult healthy volunteers were recruited and blood was collected for generation of monocyte-derived dendritic cells (moDCs) or basophil activation tests. Allergic patients were defined by >0.35 kU_A/L specific IgE levels or >3 mm wheal size in skin prick test (SPT), and any of the following clinical symptoms: allergic asthma, rhinitis, conjunctivitis, atopic dermatitis, and food allergy. Healthy volunteers had no allergic symptoms. A total of 10 donors were used in the analysis.

All patients included in the study gave written informed consent and were recruited after approved application from the Ethics Commission, Medical University of Vienna (EK Nr: 1015/2017 and EK Nr: 079/2009).

Immunoblot analysis

Supernatants from cell cultures and rsFcεRI were separated on 12% SDS minigels under reducing or non-reducing conditions and transferred on nitrocellulose or PVDF membranes by wet-transfer. Membranes were probed for FcεRIα with mouse IgG2b,K anti-human FcεRIα, clone AER-37 CRA-1 (1:1000) (BioLegend, San Diego, CA, USA) or 19-1 (2) (1:500),

followed by goat anti-mouse-HRP (Santa Cruz Biotechnologies, Dallas, TX, USA). Membranes were developed with an enhanced chemiluminescence (ECL) Western Blotting substrate (Thermo Fisher Scientific, Waltham, MA, USA) and detected by a Fusion FX imaging platform.

Mice

6-8-wk-old BALB/c male and female mice were either purchased from The Jackson Laboratory or bred in the animal facility at Boston Children's Hospital (Boston, MA). IgE deficient ($IgE^{-/-}$) mice on the *Il4raF709* BALB/c background were described previously (3). Mice were all housed in specific pathogen–free conditions according to the National Institutes of Health (NIH) and all experiments were performed with cohoused and littermate controlled cohorts that contained both genders. All animal studies were approved by the Boston Children's Hospital Institutional Animal Care and Use Committee. For passive sensitization model the following reagents were used: murine IgE-anti-DNP (D8406 clone SPE-7, Sigma-Aldrich, Merck, St. Louis, MO, USA), DNP-OVA (D-5051, Biosearch Technologies, Petaluma, CA, USA) and human rsFcεRI (laboratory of Theodore Jardetzky). For active sensitization model the following reagents were used: OVA (A7641, Sigma-Aldrich, Merck, St. Louis, MO, USA), Imject® Alum (77161, Thermo Fisher Scientific, Waltham, MA, USA) and human rsFcεRI (laboratory of Theodore Jardetzky).

Statistical analyses

All statistical analyses were performed using Prism 5 and 6 (GraphPad Software), and results are shown as mean ± SEM of the indicated number of individual data points or independent experiments (at least three). Statistical analysis was performed using Kruskal-Wallis test plus Dunn's multiple correction for comparison of two groups, 1way ANOVA test plus Tukey's multiple correction for comparison of more than three or more unmatched groups, and 2way ANOVA test plus Tukey's or Bonferroni's multiple correction for multiple comparisons between three or more groups; p values ≤ 0.05 were considered significant.

FIGURE LEGENDS

Supplementary Figure 1. TAPI-2 partially blunts sFcεRI release from MelJuso-αγ. Panel A shows MFI of bound cIgE (left) and surface FcεRIα (right) on MelJuso-αγ cells. Panel B shows sFcεRI levels detected by ELISA. Cells were loaded overnight with cIgE (1 µg/mL), and incubated with TAPI-2 (18-72 µM) for 30 minutes before addition of NP-OVA (50 µg/mL) for 2 hours. Graphs show mean ± SEM of independent experiments (n=3). 1way ANOVA tests plus Tukey's multiple correction were performed where ****p<0.0001 compared to the second condition.

Supplementary Figure 2. Chimeric FcεRI binds cIgE and internalizes after crosslinking. Analysis of receptor loading and internalization on mature bone marrow MCs by flow cytometry. Panel A shows a representative dot plot of MCs, and histogram of cIgE-loading (1 and 10 µg). Panel B shows the MFI of surface bound cIgE (left) and surface FcεRIα (right) on MCs. Cells were loaded overnight with cIgE (100 or 500 ng/mL) and BSA or NP-BSA (100 ng/mL) was added for 2 hours. Graphs show mean ± SEM of independent experiments (n=2). 1way ANOVA tests plus Tukey's multiple correction was performed where ***p<0.001 compared to the first condition.

Supplementary Figure 3. Endogenously produced and recombinant sFcεRI inhibit cIgE binding and crosslinking. Detection of bound cIgE (left) and surface FcεRIα (right) on MelJuso-ØØ/αγ/αβγ cells. Endogenous sFcεRI was harvested and purified from MelJuso cell cultures. Cells were loaded overnight with cIgE (1 µg/mL) in presence or absence of endogenous or recombinant (2.5-25 nM) sFcεRI. NP-OVA (50 µg/mL) was added for 3-5 hours. Graphs show mean ± SEM of independent experiments (n=3-6). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05, **p<0.01, and ***p<0.001 compared to the second condition.

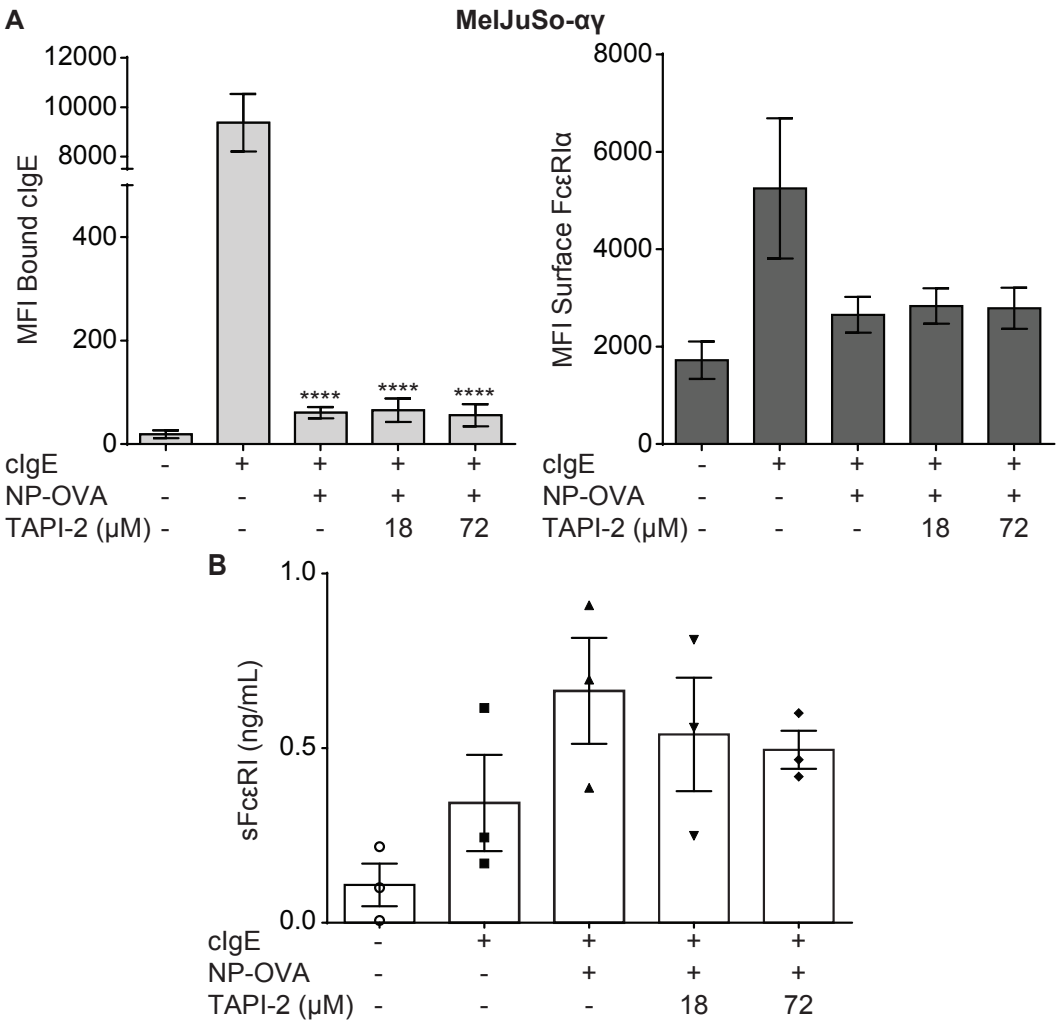
Supplementary Figure 4. sFcεRI blocks cell surface cIgE binding. Detection of bound cIgE (left) and FcεRIα (right) on MelJuso-αβγ cells by flow cytometry (sFcεRI (25 nM) and omalizumab (0.27 µM)). Graphs show mean ± SEM. Individual points represent means of independent experiments (n=3). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05, **p<0.01, and ***p<0.001, compared to cells loaded with cIgE.

Supplementary Figure 5. Gating strategy for human basophils. Detection of surface CCR3 and CD63 on basophils by flow cytometry. Representative dot plot of PBMCs isolated from healthy or allergic volunteers (n=3) gated as CCR3+/SSClow. CD63+ gate was adjusted according to donor's background. B: background.

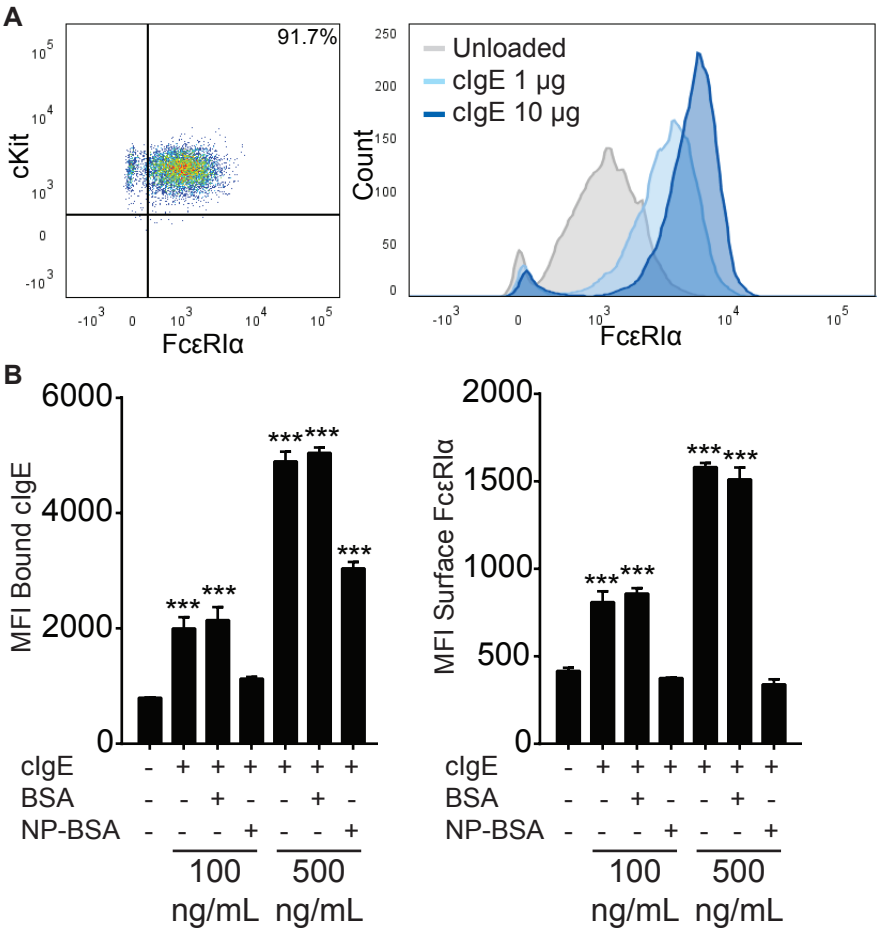
Supplementary Figure 6. sFcεRI prevents systemic anaphylaxis and improves recovery in actively-sensitized mice. Detection of recombinant sFcεRI and in MCs cultures by Western Blot (A) and analysis of core body temperature from systemically sensitized mice (B, C). Panel A shows a representative Western Blot analysis of sFcεRI from BMMCs cultures or recombinantly produced. Panel B shows temperature drops at the 45 min time point. Graphs shown mean ± SEM where individual points represent each mouse. Graphs shown mean ± SEM of each group (n=3-4 mice per group). 1way ANOVA tests plus Tukey's multiple correction were performed where *p<0.05and ****p<0.0001.

1. Garman SC, Kinet JP, Jardetzky TS. The crystal structure of the human high-affinity IgE receptor (Fc epsilon RI alpha). Annu Rev Immunol. 1999;17:973-6.
2. Fiebiger E, Tortorella D, Jouvin MH, Kinet JP, Ploegh HL. Cotranslational endoplasmic reticulum assembly of FcepsilonRI controls the formation of functional IgE-binding receptors. J Exp Med. 2005;201(2):267-77.
3. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. Nature. 1994;370(6488):367-70.

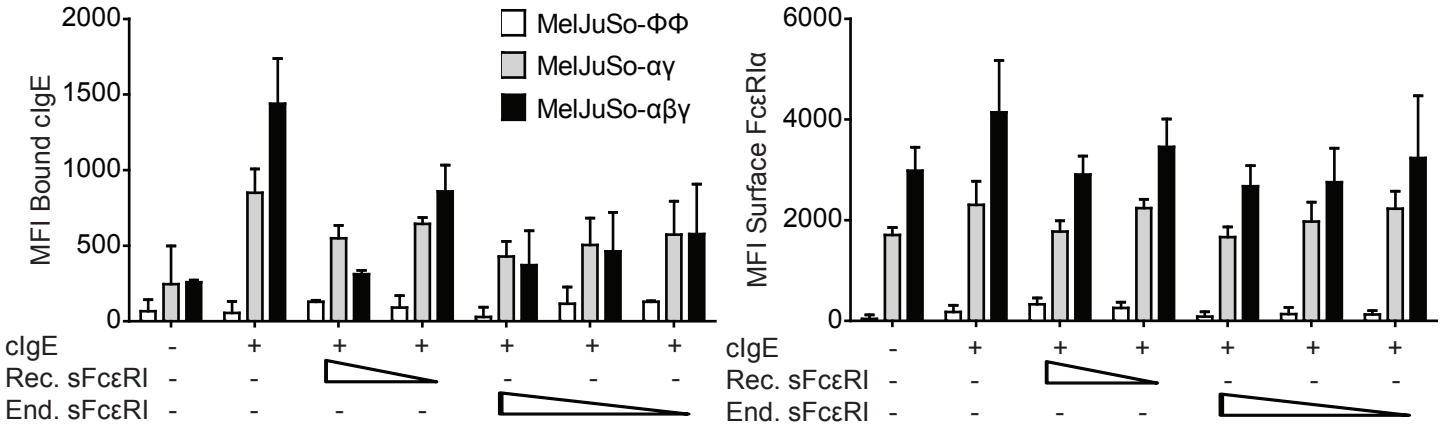
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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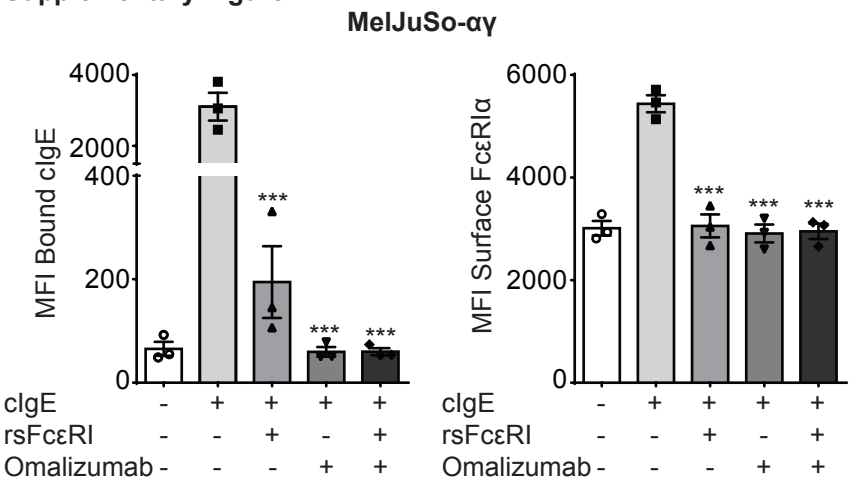

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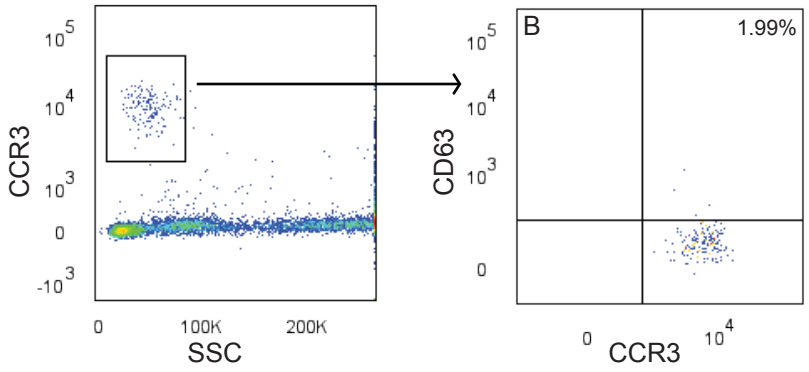

Supplementary Figure 6