Received: 29 August 2023 | Revised: 14 February 2024 | Accepted: 20 February 2024

DOI: 10.1111/all.16090

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

ORIGINAL ARTICLE

Generation of a virus-like particles based vaccine against IgE

Zahra Gharailoo[1,2,3] | Kevin Plattner[1,2,3] | Gilles Augusto[1,2,4] | Paul Engeroff[1,2] | Monique Vogel[1,2] | Martin F. Bachmann[1,2,4]

[1]Department of Immunology, University Clinic for Rheumatology and Immunology, University of Bern, Bern, Switzerland

[2]Department of Biomedical Research Bern (DBMR), University of Bern, Bern, Switzerland

[3]Graduate School for Cellular and Biomedical Sciences (GCB), Bern, Switzerland

[4]Nuffield Department of Medicine, The Jenner Institute, University of Oxford, Oxford, UK

Correspondence
Monique Vogel and Martin F. Bachmann, Department of Immunology, University Clinic for Rheumatology and Immunology, University of Bern, Bern, Switzerland.
Email: martin.bachmann@unibe.ch and monique.vogel@unibe.ch

Funding information
SNF Martin Bachmann, Grant/Award Number: 310030_185114; SNF Monique Vogel, Grant/Award Number: 310030_179165

Abstract

Background: Anti-IgE immunotherapy with monoclonal antibodies represents a breakthrough in treatment of severe allergic diseases. However, drawbacks such as short half-life and high price are not negligible. Our objective is to develop an anti-IgE vaccine based on virus-like particles (VLPs) which can induce long-lasting neutralizing IgG anti-IgE antibodies reducing allergic responses without causing intrinsic mast cell activation due to IgE cross-linking.

Methods: The vaccines were made by chemically coupling three synthetic mouse IgE-Fc fragments to plant-derived immunologically optimized $CuMV_{TT}$ VLPs. The immunogenicity of the vaccines was tested by immunizing naive or allergic mice either with the coupled vaccines or the VLP control followed by systemic or local allergen challenge.

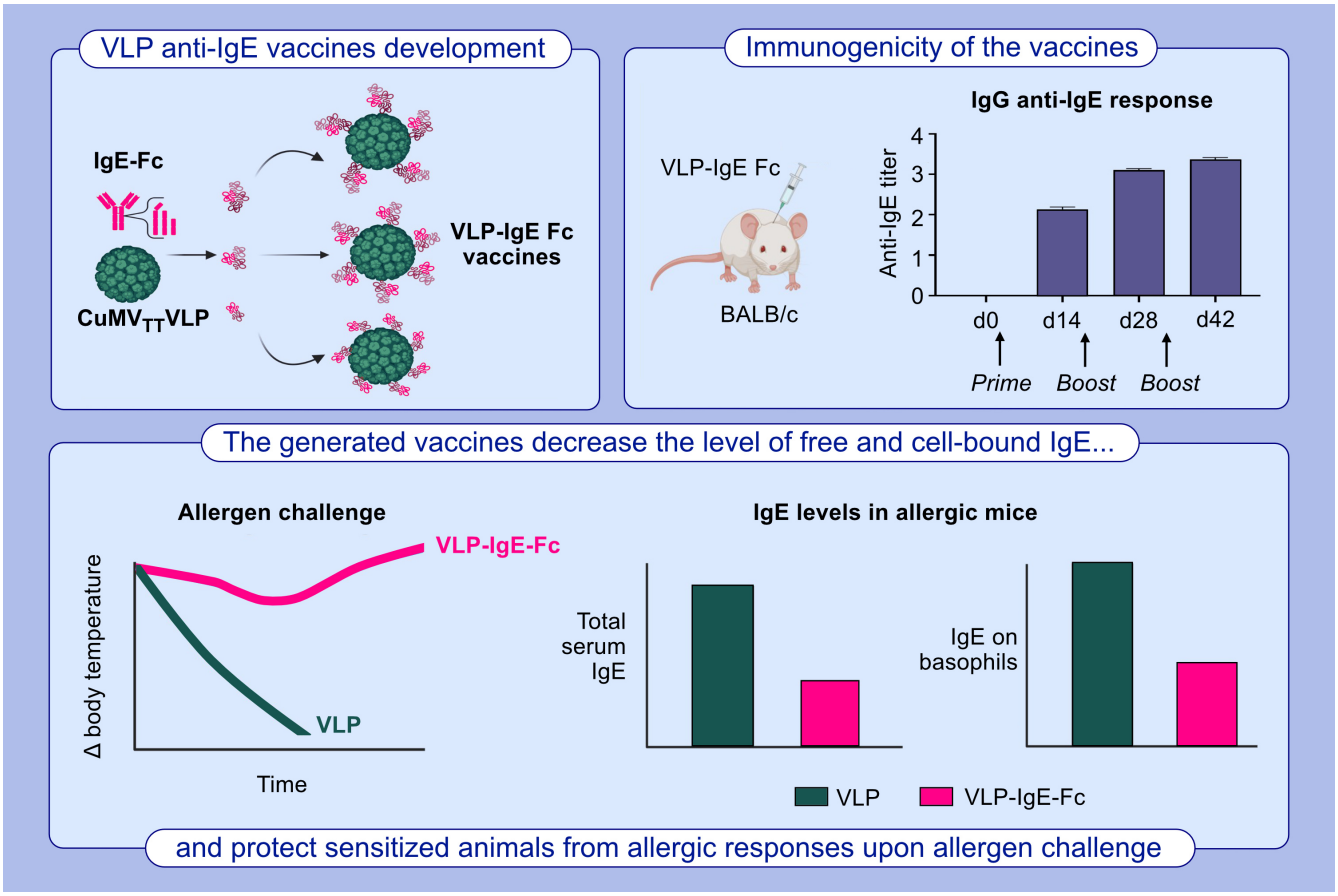
Results: Mice immunized with the vaccines exhibited high titers of anti-IgE antibodies in the sera and high levels of anti-IgE secreting plasma cells in lymphoid organs. Moreover, free IgE in serum were reduced by the induced anti-IgE antibodies; therefore, less IgE was bound to FcεRI on the surface of basophils. In line with these reduced IgE levels on effector cells after vaccination, immunized mice were protected from challenge with allergens. Importantly, despite presence of anti-IgE antibodies, no signs of acute or chronic allergic response were seen in immunized allergic mice.

Conclusion: The generated vaccines can effectively induce anti-IgE antibodies that did not cause allergic responses in sensitized mice but were able to decrease the level of free and cell bound IgE and protected sensitized animals from allergic responses upon allergen challenge.

KEYWORDS
allergens, allergy, IgE, vaccination, VLPs

1 | INTRODUCTION

The World Health Organization (WHO) has reported that allergic diseases have become one of the top three conditions that require substantial efforts for better control in the 21st century.[1] An increase in immunoglobulin E (IgE) levels is the major characteristic of allergic conditions.[2,3] IgE exhibits a short half-life of 2–3 days in serum and plays a pivotal role in type I allergic reactions also



GRAPHICAL ABSTRACT

This study aimed to develop an anti-IgE vaccine based on VLPs which can induce long-lasting neutralizing IgG anti-IgE antibodies reducing allergic responses without causing intrinsic mast cell activation due to IgE cross-linking. VLP-based anti-IgE vaccine candidates were produced by chemically coupling synthetic mouse IgE-Fc fragments to plant-virus derived CuMV$_{TT}$ VLPs. These vaccine candidates were highly immunogenic and induced protection upon allergen challenge by decreasing the level of free and cell bound IgE. Abbreviations: CuMV$_{TT}$, Cucumber mosaic virus engineered with a universal T cell epitope; Ig, immunoglobulin; VLPs, virus-like particles.

in defense against parasites.[3–7] It is structured with two identical heavy and light chains; each heavy chain consists of four constant epsilon domains (Cε1-4).[8] There are two main known receptors for IgE-Fc on different immune cells, FcεRI (high-affinity receptor) and FcεRII (CD23). Cross-linking of FcεRI-bound IgE on the surface of mast cells and basophils by allergen is a key activating signal for cellular degranulation and release of inflammatory mediators. This causes an immediate hypersensitivity response that—in its most extreme form—is capable of inducing systemic anaphylaxis with potentially fatal outcome.[9–13] In recent years, numerous therapeutic strategies were developed to block binding of IgE to its high-affinity receptor. These include monoclonal anti-IgE antibodies, Fc-fusion proteins, DARPin® proteins, and nanobodies.[14–21] Up to now, omalizumab (Xolair® Novartis) is the only clinically approved humanized anti-IgE monoclonal antibody which directly targets free IgE and disrupt its binding to both FcεRI and CD23.[14,22] While Omalizumab and other pharmacological treatments are available to address many allergic diseases, there is a need for additional therapeutic drugs with long-lasting effect and with an affordable price-tag. A vaccine against IgE would offer affordability and infrequent dosing as well as similar clinical benefits to omalizumab by lowering free IgE levels resulting in less binding of IgE to FcεRI on mast cells and basophils.[23] It has been reported previously that a peptide consisting of 76-amino acids containing parts of Cε2 and Cε3 domains (as the corresponding region which binds to FcεRI) was able to inhibit cellular activation upon passive sensitization of human skin mast cells in vivo and basophil sensitization with human IgE antibodies in vitro.[24] Another study has demonstrated that immunizing ovalbumin-sensitized rats with Cε3 domain of rat IgE framed by Cε2 and Cε4 domains of opossum IgE, conjugate or fused to a carrier protein, leads to a highly significant lowering (90%) of serum IgE levels.[25] In designing an anti-IgE vaccine, a key issue is that anti-IgE antibodies induced by vaccination may cause anaphylactic reactions in the host by cross-linking IgE bound to FcεRI on effector cells.[24,26] However, our group has demonstrated that anti-IgE autoantibodies represent a physiological pathway for the noninflammatory downregulation of serum IgE in the absence of allergic reactions.[27,28] This led us to develop an anti-IgE vaccine based on virus-like particles (VLPs) which is an established vaccine platform for inducing high antibody responses against a desired

antigen. VLPs have already been used to treat type 2 inflammation by inducing protective antibodies against allergens or against inflammatory cytokines but never against IgE.[29–33] The vaccine candidates consist of different recombinant IgE fragments coupled to cucumber mosaic virus (CuMV) particles. All candidates fulfill the essential requirements of an anti-IgE therapeutic in that they protect mice from allergic anaphylaxis by downregulating IgE levels without causing IgE-mediated inflammation.

2 | MATERIALS AND METHODS

2.1 | Gene cloning

Three constructs for IgE-Fc fragments (Cε1-Cε2-Cε3-Cε4, Cε2-Cε3-Cε4, and Cε3-Cε4 which are termed herein as Cε1-Cε4, Cε2-Cε4, and Cε3-Cε4, respectively) were designed as single genes coding each protein of interest fused to an N-terminal μ-phosphatase secretion sequence and a C-terminal 6xHis + GGCG tag for affinity purification/ VLP coupling. These sequences were codon-optimized for *Homo sapiens*, synthesized, and inserted into pTwist-CMV-BetaGlobin-WPRE-Neo vector by Twist Biosciences (San Francisco CA, USA) (Figure 1A).

2.2 | Protein expression and purification

Expression of the construct (IgE-Fc–His Tag) plasmids was amplified in XL-1 Blue electrocompetent cells and then extracted with Pure-Link™ HiPure Plasmid Filter midiprep Kit (Invitrogen, catalog K210016, Waltham, MA, USA) according to manufacturer's protocol. These synthetic constructs were transfected into Expi293F cells (Invitrogen, ThermoFisher Scientific, MA, USA) using ExpiFectatmine 293 (Gibco, ThermoFisher Scientific, MA, USA) as and expressed as described (Pub. No. MAN0007814). Purification was performed by IMAC using a HiTrap TALON crude column (Cytiva, Uppsala, Sweden). Fractions containing the IgE-Fc proteins were collected, concentrated, and buffer-exchanged to PBS using Vivaspin® 6 10 kDa MWCO spin column (Cytivia Sweden AB, Sweden).

2.3 | Generation of $CuMV_{TT}$-Cε vaccines

The production and purification of $CuMV_{TT}$ VLPs is described in Zeltins et al.[29] For the generation of $CuMV_{TT}$–Cε, three IgE-Fc fragments were conjugated to $CuMV_{TT}$ using the cross-linker SMPH (Succinimidyl 6-((beta-maleimidopropionamido) hexanoate)), (Thermo Fisher Scientific, Waltham, MA, USA) at a 10 molar excess to $CuMV_{TT}$ for 30 min at 25°C. $CuMV_{TT}$ and Cε proteins were coupled at a molar ratio 1:1 by shaking at 25°C for 3 h at 400 rpm. Unreacted SMPH and Cε proteins were removed using Amicon Ultra 0.5, 100 K (Merck Millipore, Burlington, MA, USA) (Figure 1A).

2.4 | Mice immunization and serum collection

Female naive BALB/c OlaHsd mice at age 8–12 weeks were purchased (Envigo, Horst, The Netherlands) and kept in SPF animal facility (Department of Biomedical Research, University of Bern, Bern, Switzerland) according to the guidelines of Cantonal Veterinary, Bern. The mice were immunized subcutaneously (s.c.) either with 5 μg $CuMV_{TT}$ coupled to Cε domains ($CuMV_{TT}$-Cε1-Cε4, $CuMV_{TT}$-Cε2-Cε4, and $CuMV_{TT}$-Cε3-Cε4) or with $CuMV_{TT}$ (5 μg) as control. Boosting was performed with an equal dose on Days 14 and 28, and the mice were weekly bled via tail veins until Day 42. To assess anti-IgE IgG amount, sera were isolated using Microtainer Tube (BD Biosciences, USA).

2.5 | Flow cytometry

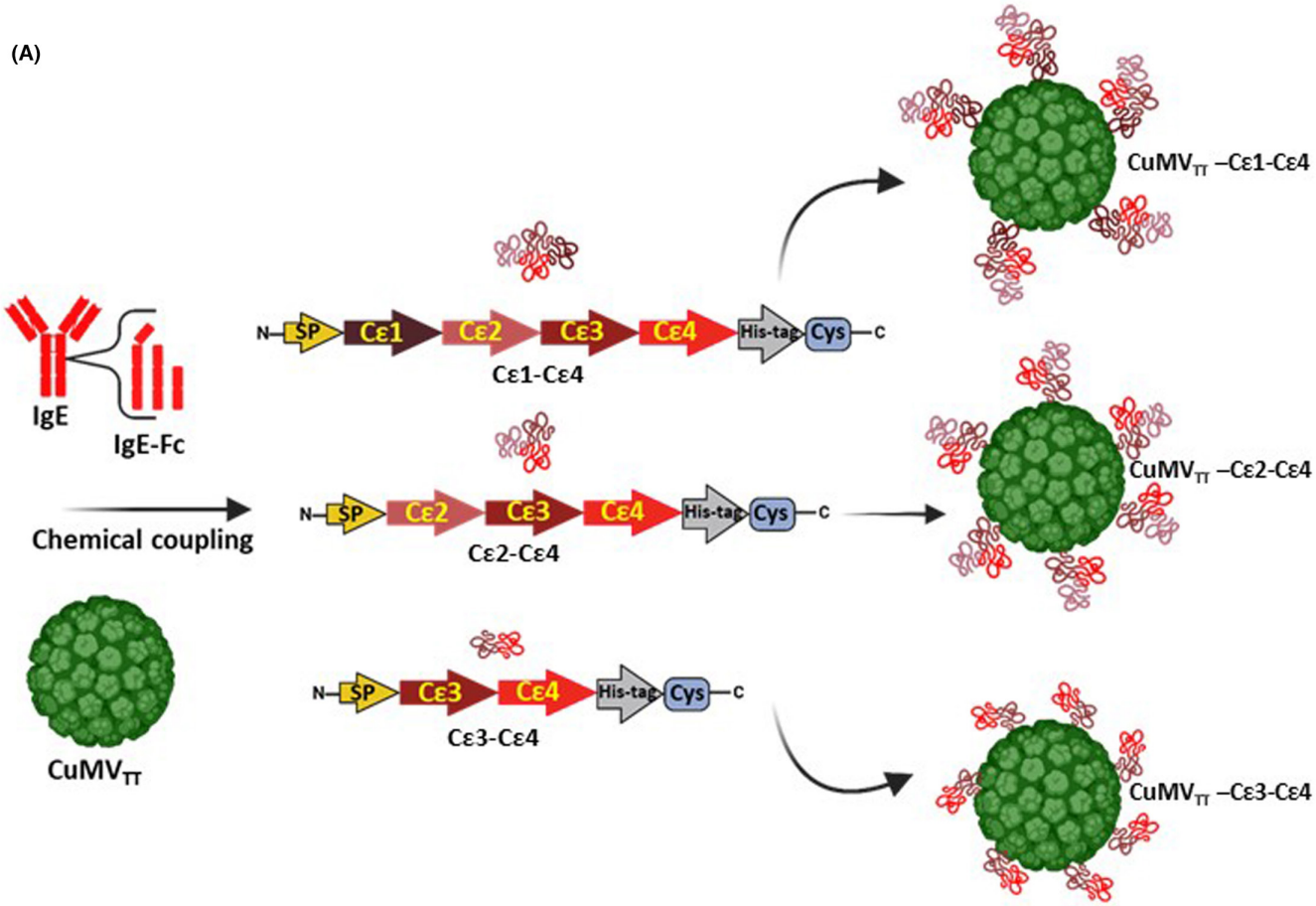
Red blood cells were lysed using ACK lysis buffer (Thermo Fisher). To prevent unspecific binding mouse Fc gamma block (BD Bioscience) was used. APC anti-mouse CD49b (clone HMa2, Biolegend) and PE anti-mouse IgE (clone RME-1, Biolegend) positive cells were defined as basophils. Flow cytometry was performed with CytoFLEX S 4 L 13C (B2-R3-V4-Y4) plus 96 DW plate loader (Beckman Coulter Life Sciences, CA, USA) and analyzed by using FLOWJO software (TreeStar Inc, Ashland, OR, USA).

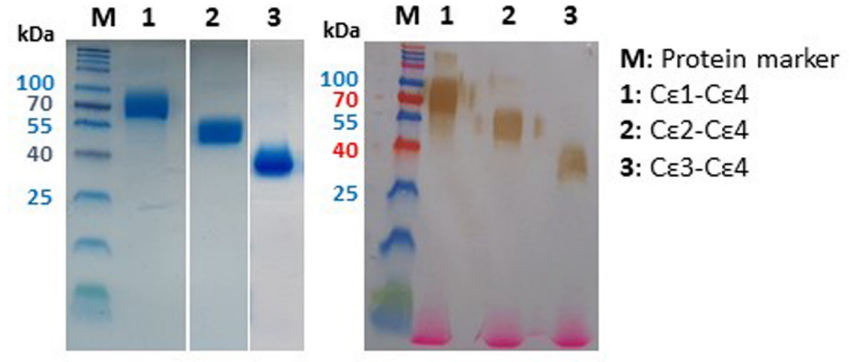
2.6 | Passive sensitization and challenge

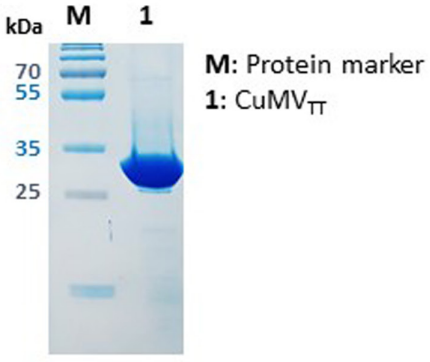
Naive mice which were immunized with $CuMV_{TT}$-Cε vaccines, intravenously (i.v.) were injected with 6 μg of IgE F127 (house-made monoclonal anti-Fel d 1 antibody). The next day, baseline body temperature was measured by using a MiniTemp rectal probe for mice (Vetronic Services Ltd, Devon, United Kingdom). IgE sensitized mice were then challenged by i.v. injection with 3 μg of Fel d 1 (major cat allergen) in PBS, and their rectal temperature was measured at 10-minute intervals for 1 h.

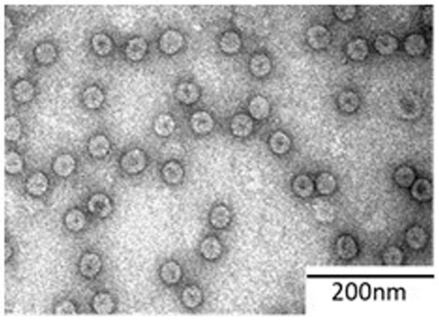
2.7 | Active sensitization and challenge

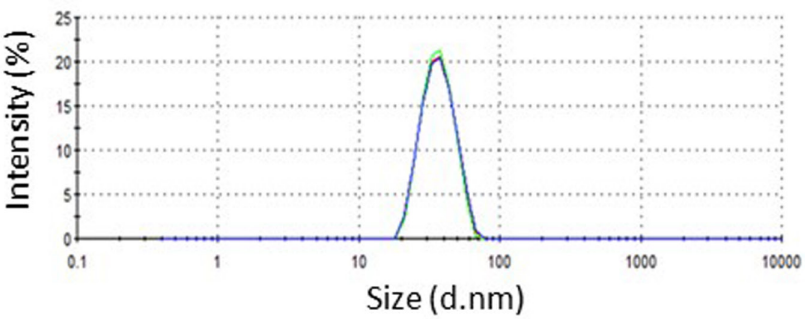
Naive mice were injected intraperitoneally (i.p.) with 0.3 μg of Fel d 1 mixed in 200 μL of 10 mg/mL Al (OH)3, Alhydrogel, referred to as Alum (InvivoGen, San Diego, Calif). To induce anaphylaxis, these mice were challenged i.v. with 3 μg Fel d 1 in 100 μL PBS. Core body temperature was measured as explained above. In the peanut allergic mice model, 5 μg peanut extract (Ara N) absorbed in 200 μL Alum administrated twice i.p. and challenge was performed by i.v. injection of 20 μg of Ara N in 100 μL PBS.











2.8 | ELISA

2.8.1 | Confirming the coupling efficiency

96-half well ELISA plates (Costar®, USA) were coated with 10 μg/mL anti-$CuMV_{TT}$ antibody (house-made monoclonal antibody from hybridoma) overnight at 4°C. After blocking with PBS-0.15% Casein for 2 h plates were incubated with different concentrations of the $CuMV_{TT}$-Cε vaccines for 1 h at RT. Next, HRP-labeled goat anti-mouse IgE (Bio-Rad) was added for 1 h at RT. The plates were developed with TMB (3,30,5,50-tetramethylbenzidine) and H_2O_2 and stopped with 1 M H2SO4 solution and OD was measured at 450 nm.

2.8.2 | Detection of mouse anti-IgE IgG and Avidity ELISA

Plates were coated with 2 μg/mL mouse IgE F127. Serially diluted sera were added following by HRP labeled goat anti-mouse IgG (Jackson Laboratory) antibodies to detect IgG. For subclasses, we used HRP labeled anti-mouse IgG1, IgG2a, and IgG2b (All BD Biosciences). To test overall affinity of the specific anti-IgE antibodies in the immunized mice sera, avidity ELISA was performed. To this end, ELISA was carried out in two parallel plates in which one plate was washed 3x with PBS-0.05% Tween-7 M urea, while the other only with PBS-0.05% Tween. Weakly bound antibodies are washed away with 7 M urea while only high-avidity antibodies are detectable at OD_{450nm}.

2.8.3 | Total serum IgE detection

Plates were coated with 2 μg/mL purified rat anti-mouse IgE (BD Biosciences). After blocking with PBS-0.15% Casein sera were added in serial dilutions. Afterwards, goat anti-mouse IgE-HRP (Bio-Rad) was used as detecting antibody. Plates were developed and stopped, and OD values were measured as described above.

2.9 | FluoroSpot assay

Fluorospot assay was run according to the manufacturer's protocol. Briefly, fluorospot plate (Mabtech, Cat no. 3654-FL) was coated with 50 μg/mL mouse IgE F127 per well, overnight at 4°C. Two million cells from bone marrow (BM) and spleen were seeded in each well, and the plate was incubated at 37°C (5% CO_2) for 20 h. Subsequently, goat anti-mouse IgG biotin primary antibody (SouthernBiotech) diluted in PBS-0.1% BSA was incubated for 2 h at RT. Then, streptavidin-550 (Mabtech) diluted in PBS- 0.1% BSA was added for 1 h at RT. Next, Fluorescence enhancer-II (Mabtech) was added for 15 min at RT. Finally, the dry plate was read at OD_{550nm} using Fluorospot reader (Mabtech IRIS).

2.10 | Skin Prick Test

The assessment of the local allergic response was conducted using the ear prick test. Mice received an intravenous of 200 μL Evans blue solution (0.5%) in PBS. Subsequently, mice were anesthetized and had their outer ear skin treated with a 20 μL drop of Fel d 1 solution (200 μg/mL in PBS). The skin of the ear was then pricked using 23G needles (0.6 mm × 25 mm) (Microlance; Becton Dickinson, Allschwill, Switzerland). The dot area was quantified using ImageJ (NIH, USA).

2.11 | Data and statistical analysis

Data were analyzed and presented as mean ± SEM using unpaired t-test, two-way or one-way ANOVA as mentioned in the figure's legend, with GraphPad PRISM 9 (GraphPad Software, Inc, San Diego, CA, USA). The values of $p < .05$ were considered statistically significant; $p < .05$ (*), $p < .01$ (**), $p < .001$ (***), $p < .0001$ (****).

3 | RESULTS

3.1 | Generation of the $CuMV_{TT}$-Cε vaccines

To generate VLP-based anti-IgE vaccine candidates, we produced three Cε fragments and coupled them to the repetitive surface of $CuMV_{TT}$ using SMPH as a chemical cross-linker[29] (Figure 1A). Since we aimed to generate antibodies that can block binding of IgE to FcεRI we produced Cε fragments (Cε1-Cε4, Cε2-Cε4, and Cε3-Cε4) containing the receptor binding epitopes (positioned in Cε3) in HEK cells. To facilitate purification, the Cε fragments were fused to a His-tag and a free Cys was added for coupling. As shown in Figure 1B,C, three bands corresponding to the sizes of each Cε fragment were identified in the purified fractions by SDS-PAGE and Western Blot. $CuMV_{TT}$ VLPs were expressed in *E. coli* and purified by sucrose gradient ultracentrifugation. The size of $CuMV_{TT}$ VLP monomers was of the expected size in SDS-PAGE analysis (Figure 1D) and electron microscopy imaging shows virus-shaped $CuMV_{TT}$ particles (Figure 1E) and the average hydrodynamic diameter (Z-Average (d.nm)) was ~35.1 nm (Figure 1F). The

FIGURE 1 Vaccines components and design. (A) Outline of the strategy to display IgE-Fc proteins on $CuMV_{TT}$ VLPs surface. (B, D) Analysis of the size and purity of the generated IgE-Fc fragments and $CuMV_{TT}$ VLPs by SDS-PAGE stained with Coomassie blue stain. (C) Western Blot developed with anti-mouse HRP-labeled IgE antibody (E) Electron microscopy (EM) of $CuMV_{TT}$ VLPs. (F) Dynamic light scattering showing VLPs' size distribution by intensity.

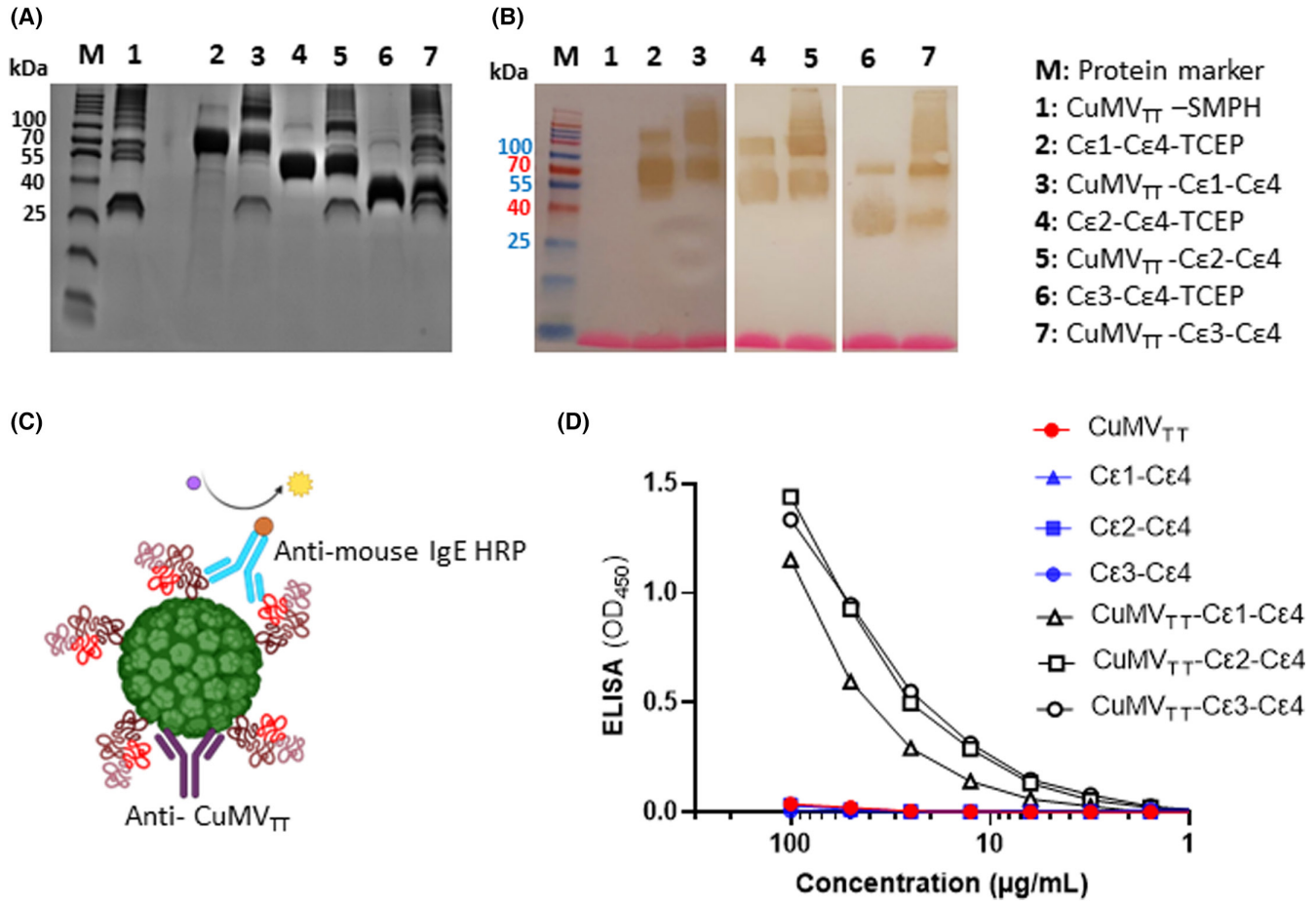


FIGURE 2 IgE-Fc fragments were efficiently coupled to CuMV$_{TT}$ VLPs. (A) Coupling reactions of IgE-Fc fragments to CuMV$_{TT}$ analyzed by SDS-PAGE using Coomassie Blue staining. (B) Western Blot developed with anti-mouse IgE-HRP antibody confirming efficient incorporation of IgE-Fc fragments into CuMV$_{TT}$ VLPs. (C) Schematic view of sandwich ELISA setting. (D) Sandwich ELISA for assessing binding of anti-CuMV$_{TT}$ monoclonal antibody to CuMV$_{TT}$-Cε vaccines. OD450 values are displayed for serially diluted different vaccines, CuMV$_{TT}$ and Cε proteins as negative controls.

coupling efficiency of Cε proteins to CuMV$_{TT}$ was analyzed by SDS-PAGE and Western Blot (Figure 2A,B). We observed that all three Cε-proteins efficiently couple to CuMV$_{TT}$ VLPs. To confirm the correct folding of the Cε fragments displayed on the surface of CuMV$_{TT}$, a sandwich ELISA was performed in which an anti-CuMV$_{TT}$ as capture antibody and an anti-IgE monoclonal antibody was used for detection (Figure 2C). The results showed that in all three vaccines, Cε proteins are recognized by anti-IgE antibodies indicating correct folding of IgE fragments on the VLP whereas no signal was observed with Cε proteins and/or CuMV$_{TT}$ alone (Figure 2D).

3.2 | Immunization with CuMV$_{TT}$-Cε vaccines induces IgG anti-IgE antibodies

To test the immunogenicity of the vaccine candidates, naive mice were immunized with CuMV$_{TT}$-Cε vaccines and CuMV$_{TT}$ as control on Day 0 and then boosted on Days 14 and 28 (Figure 3A). Our data demonstrate that Cε proteins coupled to VLPs induce high IgE-specific serum IgG responses. As shown in (Figure 3B) IgG anti-IgE antibody titers were substantially increased 14 days after booster immunizations with CuMV$_{TT}$-Cε while, immunization with CuMV$_{TT}$ induced no antibody response. Interestingly, the CuMV$_{TT}$-Cε2-Cε4 and CuMV$_{TT}$-Cε3-Cε4 vaccines induced higher levels of IgG antibodies belonging to all three subclasses compared to those generated by CuMV$_{TT}$-Cε1-Cε4 (Figure S1).

The avidity of IgG anti-IgE antibodies induced by CuMV$_{TT}$-Cε vaccine candidates was also examined for sera isolated on Day 42. To this end, we performed a modified ELISA using 7 M Urea to remove the low affinity antibodies. As shown in Figure 3C, high-avidity IgG antibodies against IgE were present in high amounts in sera from mice immunized with all three vaccine candidates. Therefore, in addition to high antibody titers, CuMV$_{TT}$-Cε vaccines induce antibodies of high avidity to IgE. Furthermore, to characterize induction of plasma cells upon CuMV$_{TT}$-Cε immunization, anti-IgE secreting plasma cells were quantified in bone marrow and spleen. To this end, femur and tibia bones as well as spleens were dissected 14 days after the last immunization and analyzed for the presence of IgG anti-IgE secreting plasma cells. As shown in Figure 3D,E, anti-IgE secreting plasma cells were detected in

both organs. On average, ~48 IgG anti-IgE secreting plasma cells were quantified per 2 million seeded bone marrow cells while this number was three times less in the spleen of all three immunized mice groups. Overall, these results show that immunization with $CuMV_{TT}$-Cε is efficient in inducing a systemic humoral immune response.

3.3 | Immunization with $CuMV_{TT}$-Cε vaccines block anaphylaxis upon challenge in passive IgE sensitization

Considering that anti-IgE antibodies can control IgE levels in the serum, we assessed whether immunized mice are protected from allergen challenge after passive sensitization with allergen-specific monoclonal IgE antibodies. Therefore, mice immunized at Days 0 and 14 with $CuMV_{TT}$-Cε vaccines as well as $CuMV_{TT}$ and were challenged with Fel d 1 one day after passive sensitization monoclonal anti-Fel d 1 IgE (Figure 4A). Strikingly, mice vaccinated with $CuMV_{TT}$-Cε were shielded from allergic reactions upon exposure to Fel d 1. In contrast, mice vaccinated with $CuMV_{TT}$ exhibited severe allergic reactions. (Figure 4B,C). These data demonstrate that $CuMV_{TT}$-Cε immunization increases protection against allergen challenge in a passive sensitization model by inducing anti-IgE antibodies.

As an important safety aspect, immunized mice injected i.v. with monoclonal IgE antibodies did not show any signs of anaphylaxis after exposure to high levels of IgE antibodies despite presence of high levels of IgE-specific IgG, indicating that the anti-IgE antibodies induced by vaccination are less anaphylactogenic than potentially expected.

3.4 | $CuMV_{TT}$-Cε vaccination induces IgG anti-IgE response in Fel d 1 and peanut allergic mice

Next, we investigated the immunogenicity of the $CuMV_{TT}$-Cε vaccines in mice previously sensitized with either Fel d 1 (cat allergy)[34,35] or peanut extract (Ara N, peanut allergy)[32,36–38] in Alum by weekly measuring IgG anti-IgE after vaccination (Figures 5B and 6B). Vaccination of allergic mice with all three vaccines caused induction of anti-IgE IgG antibodies from Day 21 after the second immunization with $CuMV_{TT}$-Cε1-Cε4 and from Day 14 after only one immunization with $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4. Additionally, in Fel d 1 allergic mice specific IgG anti-IgE secreting plasma cells were quantified in bone marrow and spleen on Day 42. Interestingly, while the number of IgG secreting plasma cells in bone marrow of $CuMV_{TT}$-Cε1-Cε4 and $CuMV_{TT}$-Cε2-Cε4 immunized mice groups were 5 times more than in spleen, those numbers were still less than in $CuMV_{TT}$-Cε3-Cε4, the smallest vaccine candidate (Figure 5C,D). As shown, on average equally ~38 secreting cells per 2 million seeded cells were quantified in both bone marrow and spleen of $CuMV_{TT}$-Cε3-Cε4 immunized group. These findings indicate the strongest capacity of $CuMV_{TT}$-Cε3-Cε4 and to a lesser extent $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε1-Cε4 to induce anti-IgE IgG antibodies in allergic mice.

As seen in the immunized mice injected with monoclonal IgE antibodies, immunization of sensitized, allergic mice did not result in any signs of disease, such as weight loss or unhealthy appearance, representing a key safety feature. Thus, vaccination of allergic mice against IgE does not cause any signs of disease and tolerability issues in two different models of allergy.

3.5 | $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4 vaccinations lower levels of serum and basophil surface IgE in allergic mice

We next aimed to assess how the induced anti-IgE antibody responses affected total IgE levels in the allergic mice. To establish the system, we measured total IgE levels in Fel d 1 allergic mice serum, plus surface IgE levels on blood basophils in Fel d 1 allergic mice (gated as $CD49b^+IgE^+$) 2 weeks after sensitization with Fel d 1 (Figure S2A). Figure S2B shows elevated basophil surface IgE levels as pseudo color plots and anti-IgE mean fluorescence intensity in Figure S2C, confirming allergen sensitization compared to the naive mice. The same was observed for total IgE levels in Fel d 1 sensitized mice (Figure S2D). We next investigated the impact of anti-IgE antibodies on clearance of serum and basophils surface IgE. Therefore, the sensitized mice were immunized with either $CuMV_{TT}$-Cε vaccines or $CuMV_{TT}$ as control 2 weeks after sensitization (Figure 5A). Basophil surface IgE and total serum IgE levels were assessed after booster immunization on Day 28. $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4 immunizations caused clearance of IgE in both serum and on the surface of basophils. In contrast, this was not observed in $CuMV_{TT}$-Cε1-Cε4 or $CuMV_{TT}$ immunized group (Figure 5E–G). These results showed that actively induced IgG anti-IgE antibodies upon vaccination are physiologically effective to downregulate free and cell-bound IgE, further indicating that $CuMV_{TT}$-Cε1-Cε4 is the least effective vaccine candidate.

3.6 | $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4 vaccinations protect mice from active systemic anaphylaxis

Given that $CuMV_{TT}$-Cε immunization protects from passive anaphylaxis, we next investigated whether anti-IgE vaccination protects against active sensitization to the allergen. To this end, we started immunization with $CuMV_{TT}$-Cε vaccines or $CuMV_{TT}$ on Day 14 following induction of active sensitization with Fel d 1 or Ara N absorbed in Alum (Figure 7A,D). These mice then were challenged i.v. with the mentioned allergens, and systemic anaphylaxis was assessed by measuring body core temperature. As shown in Figure 7B,C,E,F, $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4 immunized mice were protected from the allergen challenge whereas this was not the case when mice were immunized with control $CuMV_{TT}$ or $CuMV_{TT}$-Cε1-Cε4. To determine whether these systemic protections extend to local allergen protection, we sensitized mice and vaccinated one group with $CuMV_{TT}$ and another group with $CuMV_{TT}$-Cε3-Cε4. Ear prick tests showed

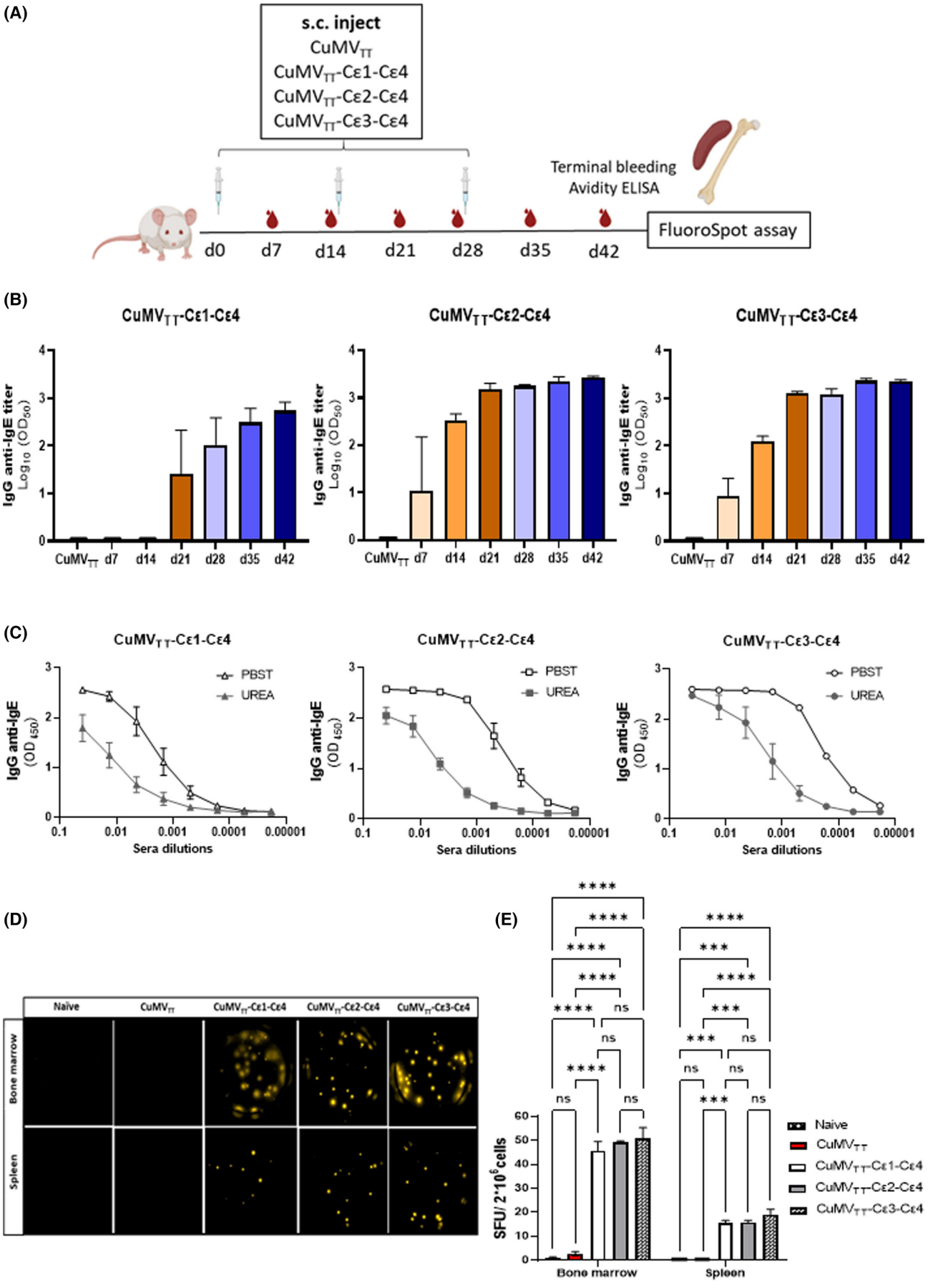


FIGURE 3 Immunization with CuMV$_{TT}$-Cε vaccines induce IgG anti-IgE response. (A) Schematic overview of the immunization regimen and specimen collections for ELISA and fluoroSpot assays. (B) IgG anti-IgE titers of immunized mice groups measured by ELISA at different time points. (C) Avidity of the induced IgG anti-IgE antibodies at Day 42. (D) Representative image of fluorospot assays showing IgG anti-IgE secreting plasma cells in spleen and bone marrow samples. (E) Bone marrow and spleen Spot Forming Units out of 2 million cells seeded in each well. statistical analysis was performed (mean ± SEM) using Two-way ANOVA: $p < .0001$ (****), $p < .001$ (***), ns, not significant, $n = 3$/group.

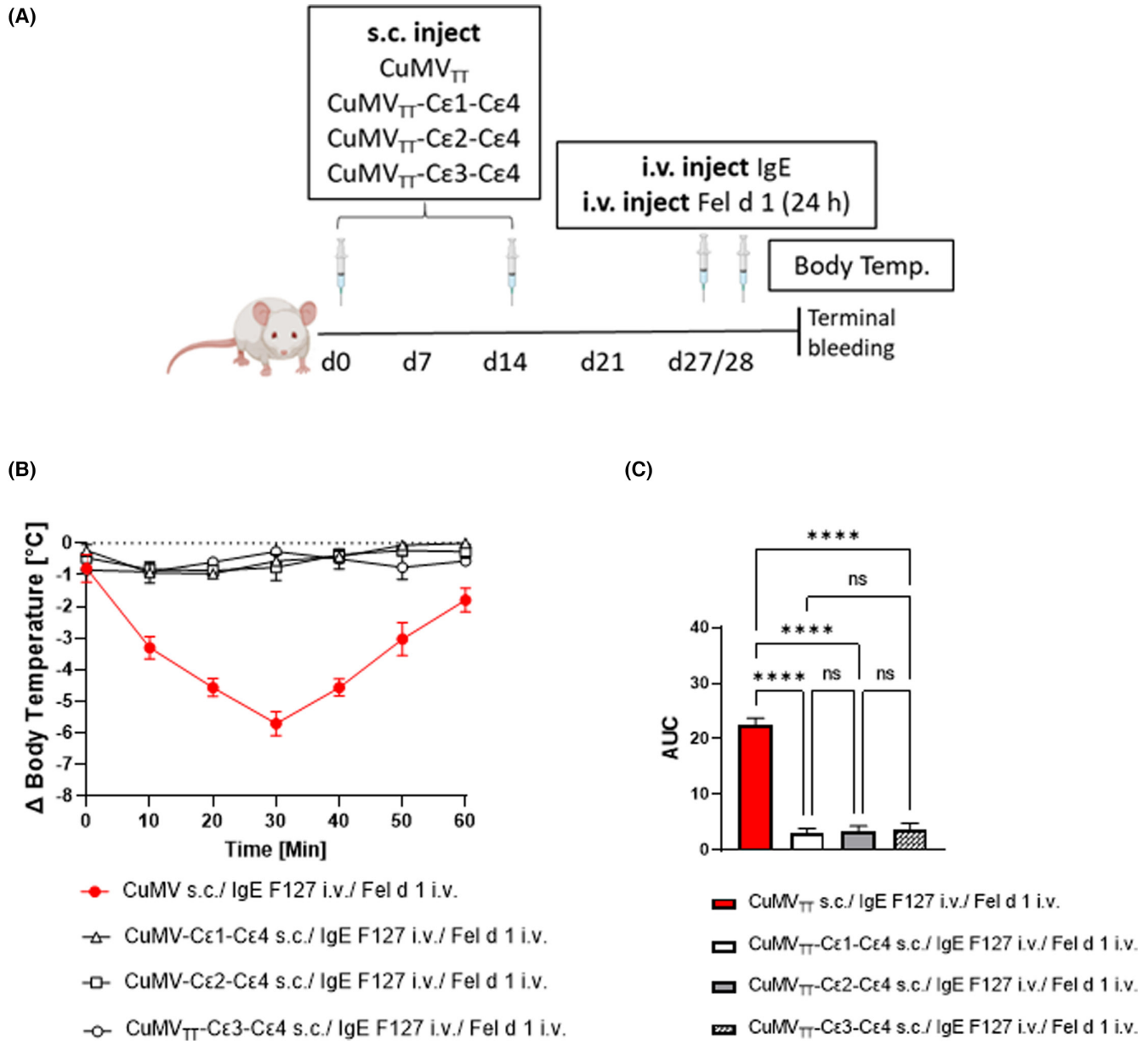


FIGURE 4 CuMV$_{TT}$-Cε immunization protects IgE sensitized mice from systemic anaphylaxis. (A) Schematic overview of immunization regimen and allergen challenge; (B) Assessment of body core temperature at 10-minute intervals upon challenge with Fel d 1 after passive sensitization with IgE F127; (C) Area under the curve upon exposure to Fel d 1. Statistical analysis was performed (mean ± SEM) using one-way ANOVA: $p < .0001$ (****), ns, not significant; $n = 3$/group from at least two independent experiments.

a significant reduction in vascular leakage in mice vaccinated with CuMV$_{TT}$-Cε3-Cε4 compared to control CuMV$_{TT}$ mice (Figure 8).

Overall, these results show how immunization with CuMV$_{TT}$-Cε induce therapeutically effective humoral immune response.

4 | DISCUSSION

IgE antibodies play a pivotal role in the allergic cascade. Reduced levels of serum-free IgE have both direct and indirect effects on

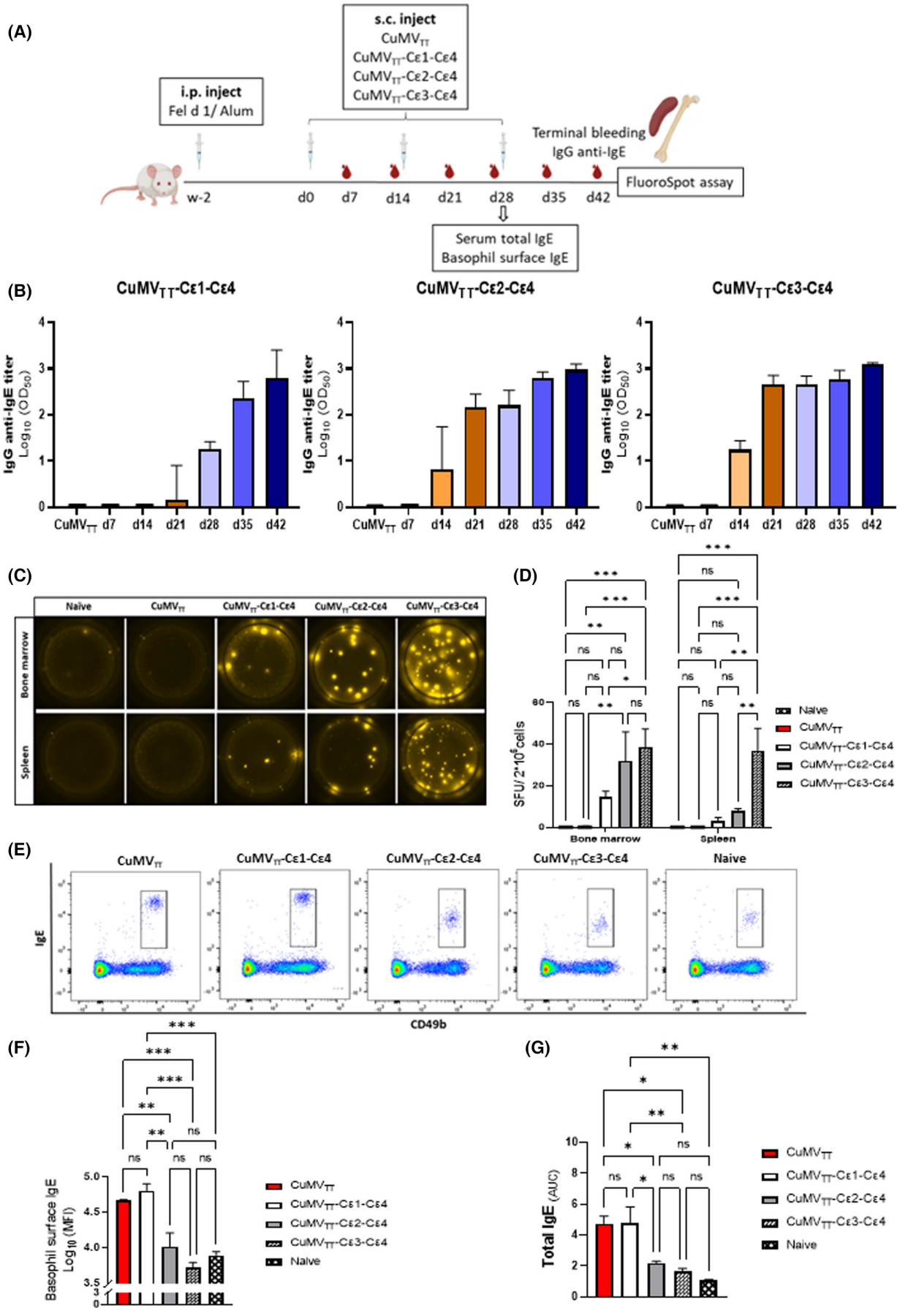


FIGURE 5 $CuMV_{TT}$-Cε immunization induces IgG anti-IgE response in Fel d 1 allergic mice and reduces serum and basophil surface IgE. (A) Schematic overview of active sensitization followed by immunization regimens and sample collections for ELISA, Fluorospot and Flow cytometry assays; (B) IgG anti-IgE titers of immunized mice groups measured by ELISA at OD50; (C) Representative pictures of the fluorospot assay showing IgG anti-IgE secreting plasma cells in spleen and bone marrow samples. (D) Bone marrow and spleen Spot Forming Units out of 2 million cells seeded in each well. Statistical analysis was performed (mean ± SEM) using two-way ANOVA: $p < .001$ (***), $p < .01$ (**), $p < .05$ (*), ns: not significant, $n = 3$/group. (E) Illustration of flow cytometry pseudo color plots showing anti-IgE staining versus anti-CD49b staining intensity. (F) Mean (±SEM) of IgE mean fluorescence intensity (MFI) on basophils of naive and Fel d 1 sensitized mice after immunization; (G) Area under the curve of ELISA plot for total serum IgE levels in naive and immunized mice measured at OD450. Statistical analysis was performed (mean ± SEM) using one-way ANOVA: $p < .05$ (*), $p < .01$ (**), $p < .001$ (***), ns: not significant, $n = 3$/group from 2 independent experiments.

allergic inflammation. It directly alleviates allergy symptoms by decreasing mast cell and basophil degranulation and the subsequent release of inflammatory mediators due to reduced cell-bound IgE. Moreover, less free IgE in the serum indirectly lowers allergic reactions by downregulating the expression of the high-affinity IgE receptor (FcεRI) on mast cells and dendritic cells.[39,40] Treatment of allergies with monoclonal anti-IgE antibodies is highly effective but there are some drawbacks in this approach including the limited half-life of these antibodies, requiring high and frequent dosing and thus high production costs.[41] Moreover, not all patients respond to omalizumab.[42] Therefore, developing a novel type of anti-IgE therapeutic vaccine, which can overcome these issues of mAbs, by inducing longer-lasting neutralizing anti-IgE antibodies would be a beneficial therapeutic method for allergic disorders. In the current study, we designed and tested three vaccine candidates against IgE. It has been previously shown that Cε3 domain is the main region of IgE interacting with FcεRI causing cellular activation in the context of allergy.[43] Herein, all three IgE-Fc fragments used in the candidate vaccines contained the Cε3 domain. We could show that immunization with $CuMV_{TT}$-Cε2-Cε4 and $CuMV_{TT}$-Cε3-Cε4 effectively induced anti-IgE IgG antibodies in prophylactic and therapeutic mouse models. Previously, several studies have analyzed the potential of IgE-derived peptide-based vaccines. Wang et al chemically coupled a 23 amino acid-peptide derived from Cε3 domain to a carrier keyhole limpet hemocyanin (KLH) and have shown that this vaccine induced strong anti-IgE responses; however, a strong adjuvant like CFA/IFA was required.[44] Peng et al have generated a vaccine against IgE by conjugating three synthetic IgE peptides derived from human Cε3 domain to a carrier protein HbsAg.[41] Another study has assessed the potential of a chimeric IgE vaccine, made up by rat IgE Cε3 domain flanked by Cε2 and Cε4 of opossum as an evolutionarily distant mammal, stabilizing the construct and introducing T helper cell epitopes.[25] In comparison with these previous vaccine candidates, our anti-IgE VLP-based vaccines have several advantages. $CuMV_{TT}$ VLPs contain ssRNA as the self-adjuvant which can enhance IgG immune response via TLR7/8 activation without application of any additional adjuvant.[29,45] Moreover, our $CuMV_{TT}$-VLP contains a universal tetanus specific T-cell epitope which allows to enhance Th-cell-dependent IgG responses, in particular, in the presence of pre-existing peptide-specific Th cells in pre-immune individuals. We have previously demonstrated that $CuMV_{TT}$ can deliver robust antibody response against cytokines causing chronic inflammatory diseases, aggregates causing degenerative diseases, and allergy.[29] In addition, our candidates are based on entire IgE domains, enabling induction of high-affinity antibodies. Indeed, all three vaccine candidates were able to induce the generation of strong anti-IgE antibody responses of high avidity. Interestingly, the candidates displaying 2–3 domains (Cε2-4 and Cε3-4) induced the highest antibody responses and were able to reduce serum and basophil surface IgE.[26]

There is one "elephant in the room," when it comes to the potential applicability of this vaccine as a novel anti-IgE therapy, regarding the question of side effects as anti-IgE antibodies can cross-link FcεRI-bound IgE, potentially causing degranulation and allergy symptoms. The success of Omalizumab and next generation of anti-IgE antibodies such as Ligelizumab is in part based on the fact that these clones fail to cross-link receptor bound IgE.[46] The risk of a vaccine to induce IgG antibodies that cross-link receptor bound IgE appears to be significant given that there is an induced polyclonal anti-IgE response. However, we have never observed any side effects in our mice, even in the presence of high anti-IgE titers in allergic mice. The absence of tolerability issues may be consistent with the observation that humans and mice present surprisingly high levels of natural anti-IgE antibodies and IgE:IgG immune complexes.[28] Mechanistically, we have shown that that as soon as the IgE forms an immune complex, it becomes non-anaphylactogenic and gets rapidly eliminated from the serum.[27,47] Another important aspect to consider is the kinetics of an allergic reaction. The slow and gradual rise of serum anti-IgE antibodies over time after vaccination is unlikely to be sufficient to trigger allergic effector cells because they generally respond rapidly to the sudden presence of cross-linking antigen. Two vaccines (Cε2-4 and Cε3-4) were able to induce protection in allergen sensitized mice while this was not the case for the third candidate (Cε1-4). This disparity could potentially be due to the reduced capability of anti-IgE IgG antibodies generated against Cε1-4 to recognize FcεRI-bound IgE, which adopts an open conformation, in contrast to the closed form observed in soluble IgE.[48] Indeed, vaccine-induced antibodies may have different functional activities regarding the epitope specificities. To this end, antibodies generated against Cε2-4 and Cε3-4 may recognize epitopes in the Cε domain resulting in increased complexation leading to enhanced serum clearance and reduced FcεRI sensitization. Indeed, previous studies have shown that IgEs can adopt different conformations upon binding to anti-IgE antibodies that either prevent or enhance the

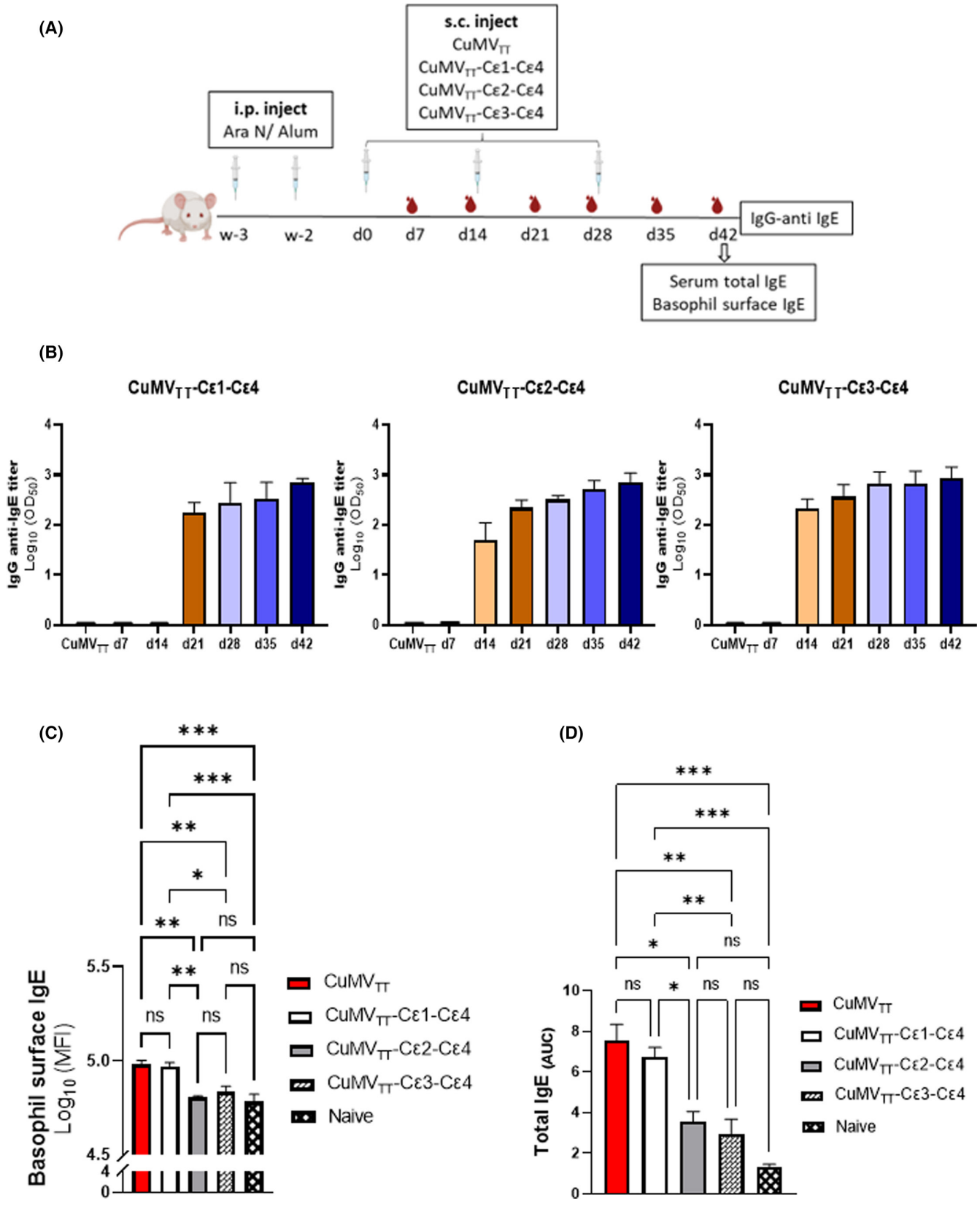


FIGURE 6 $CuMV_{TT}$-Cε immunization induce IgG anti-IgE response in peanut allergic mice and reduce serum and basophil surface IgE. (A) Schematic overview of active sensitization followed by immunization regimens and sample collections for ELISA and Flow cytometry assays. (B) IgG anti-IgE titers of individual immunized mice measured by ELISA. (C) Mean (±SEM) of IgE mean fluorescence intensity (MFI) on basophils of naive and Ara N sensitized mice after immunization. (D) Area under the curve of ELISA plot for total serum IgE levels in naive and immunized mice measured at OD450. Statistical analysis was performed (mean ± SEM) using one-way ANOVA: $p < .001$ (***), $p < .01$ (**), $p < .05$ (*); ns, not significant; $n = 4$/group.

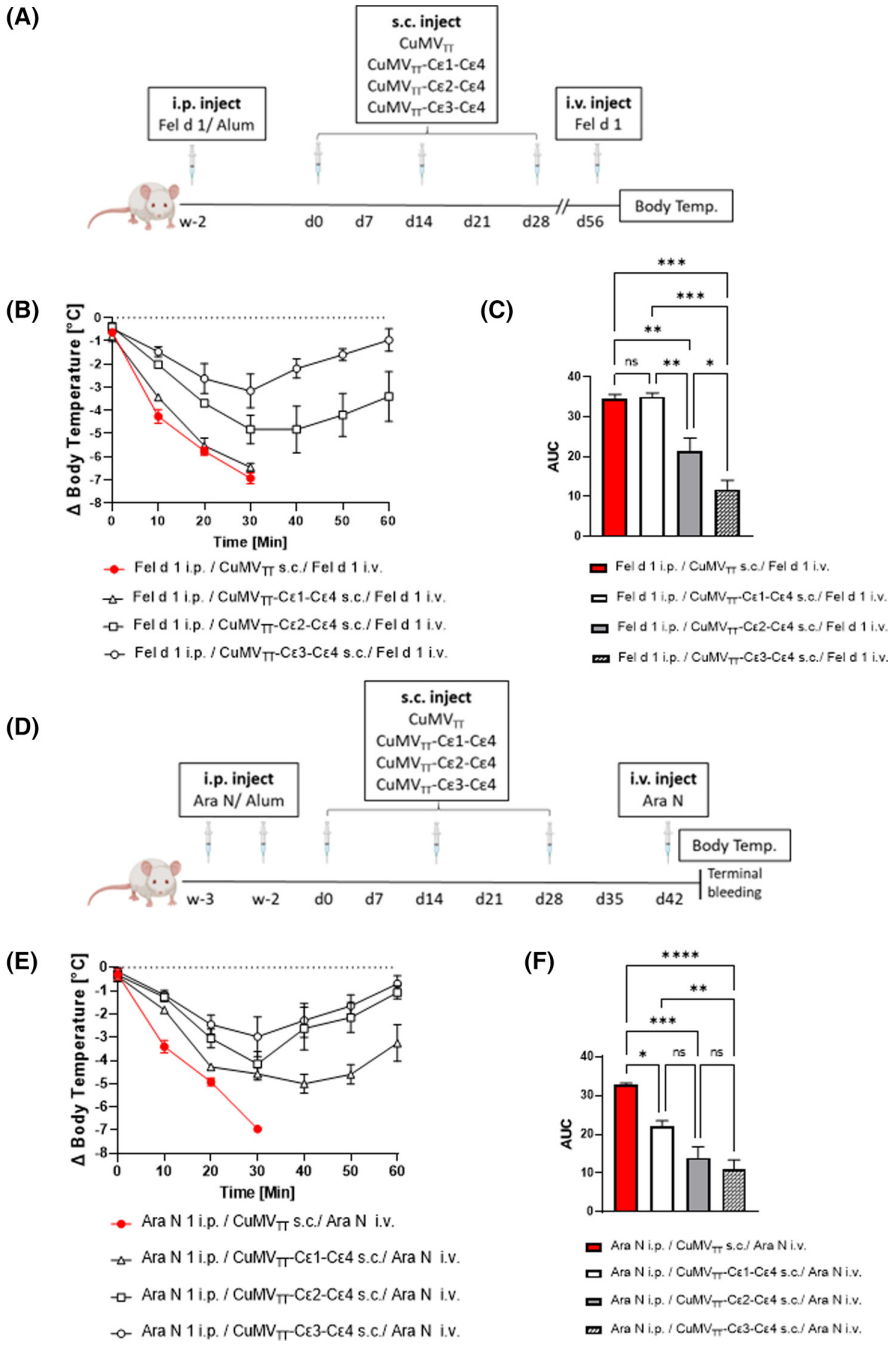


FIGURE 7 CuMV$_{TT}$-Cε2-Cε4 and CuMV$_{TT}$-Cε3-Cε4 immunization protects mice from active systemic anaphylaxis. (A,D) Schematic overviews of active sensitization with Fel d 1 and Ara N prior to immunization followed by allergen challenge. (B,E) show the drop in body core temperature at 10-minute intervals upon challenge with Fel d 1 and/or Ara N respectively. (C,F) Area under the curve of the Fel d 1 and/ or Ara N challenges, respectively. Statistical analysis was performed (mean ± SEM) using one-way ANOVA: .0001 (****), $p < .001$ (***), $p < .01$ (**), $p < .05$ (*); ns, not significant; $n = 3$ for Fel d 1 group and 4 for Ara N group from at least two similar experiments.

binding to the two receptors, FcεRI and CD23.[49] This is also in line with our previous finding that IgE complexed with IgG is generally noninflammatory.[50] Alternatively, the lower coupling efficiency resulting in lower epitope density of the large domain may offer an explanation. Lower coupling efficiency results in lower epitope density, which in causes reduced B-cell responses, in particular in the presence of partial B-cell tolerance, which may be induced by elevated levels of free IgE in allergic mice.[51]

In summary, our study demonstrates the efficacy of VLP-based vaccines against IgE, eliciting high-affinity antibodies and providing protection in sensitized mice against allergens. Notably, no adverse reactions were observed in mice, addressing concerns about IgE receptor cross-linking. Additionally, the varying effectiveness of vaccine candidates emphasizes the critical role of epitope specificity in modulating FcεRI sensitization. These results highlight the promising potential of VLP-based vaccines as a novel therapeutic strategy for allergic disorders, characterized by improved safety and efficacy profiles.

AUTHOR CONTRIBUTIONS

ZG designed, performed, acquired data, and interpreted experiments. KP, PE helped perform experiments. GA designed the vector for the production of the Cε fragments. ZG wrote the manuscript. MV, PE, and MFB revised the manuscript. MV and MFB supervised the study. All authors read and approved the final manuscript.

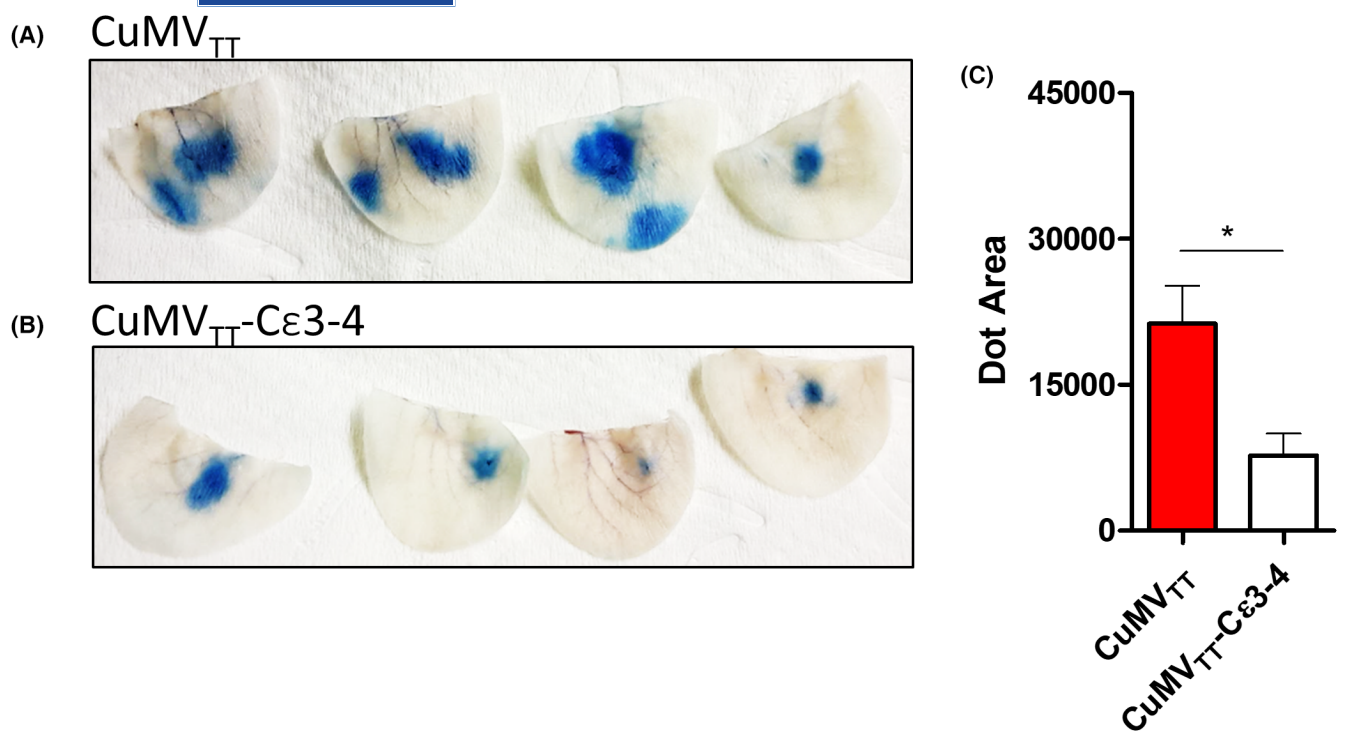


FIGURE 8 CuMV$_{TT}$-Cε3-Cε4 immunization protects Fel d 1 allergic mice from local allergen challenge. Mice were i.v. injected with Evans Blue Dye and an ear prick challenge was done with Fel d 1. Shown is the vascular leakage of Evans Blue Dye into ears of (A) CuMV$_{TT}$ or (B) CuMV$_{TT}$-Cε3-Cε4 mice, 30 min after challenge. (C) Shown is the dot area quantification of the two groups done by Image J profile analysis.

ACKNOWLEDGMENTS

We acknowledge Marianne Zwicker for technical assistance. Open access funding provided by Universitat Bern.

FUNDING INFORMATION

This project was supported by the funding from the following grants: SNF grant 310039_185114 to MB; SNF grant 310030_179165 to MV.

CONFLICT OF INTEREST STATEMENT

M. F. Bachmann is a board member of HypoPet AG. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Zahra Gharailoo https://orcid.org/0009-0006-5373-1300
Kevin Plattner https://orcid.org/0000-0002-2414-9593
Gilles Augusto https://orcid.org/0000-0001-6509-0148
Paul Engeroff https://orcid.org/0000-0002-3409-512X
Monique Vogel https://orcid.org/0000-0002-5219-4033
Martin F. Bachmann https://orcid.org/0000-0003-4370-2099

REFERENCES

1. Wirl C, Puklová V. Prevalence of asthma and allergies in children (fact sheet No 3.1). European Environment and Health Information System 1–4 2007.
2. Pate MB, Smith JK, Chi DS, Krishnaswamy G. Regulation and dysregulation of immunoglobulin E: a molecular and clinical perspective. *Clin Mol Allergy*. 2010;8:1-13.
3. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. *Nat Rev Immunol*. 2014;14:247-259.
4. Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity with gamma-E-globulin antibody. *J Immunol*. 1966;97:840-853.
5. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol*. 2008;8:205-217. doi:10.1038/nri2273
6. Platts-Mills TAE, Heymann PW, Commins SP, Woodfolk JA. The discovery of IgE 50 years later. *Ann Allergy Asthma Immunol*. 2016;116:179-182.
7. Lawrence MG, Woodfolk JA, Schuyler AJ, Stillman LC, Chapman MD, Platts-Mills TAE. Half-life of IgE in serum and skin: consequences for anti-IgE therapy in patients with allergic disease. *J Allergy Clin Immunol*. 2017;139:422-428.e4.
8. Sutton B, Davies A, Bax H, Karagiannis S. IgE antibodies: from structure to function and clinical translation. *Antibodies*. 2019;8:19.
9. Von Bubnoff D, Novak N, Kraft S, Bieber T. The central role of FcεRi in allergy. *Clin Exp Dermatol*. 2003;28:184-187.
10. Holgate ST, Djukanović R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. *Clin Exp Allergy*. 2005;35:408-416.
11. Greer AM, Wu N, Putnam AL, et al. Serum IgE clearance is facilitated by human fc e RI internalization find the latest version: serum IgE clearance is facilitated by human fc ε RI internalization. *J Clin Invest*. 2014;124:1187-1198.
12. Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. *Nat Rev Immunol*. 2007;7:365-378.
13. Gounni AS. The high-affinity IgE receptor (FcεRI): a critical regulator of airway smooth muscle cells? *Am J Physiol Lung Cell Mol Physiol*. 2006;291:312-321.

14. Presta LG, Lahr SJ, Shields RL, et al. Humanization of an antibody directed against IgE. *J Immunol*. 1993;151:2623-2632.
15. Wan M, Shiau FY, Gordon W, Wang GY. Variant antibody identification by peptide mapping. *Biotechnol Bioeng*. 1999;62:485-488.
16. Brightbill HD, Jeet S, Lin Z, et al. Antibodies specific for a segment of human membrane IgE deplete IgE-producing B cells in humanized mice. *J Clin Invest*. 2010;120:2218-2229.
17. Chu SY, Horton HM, Pong E, et al. Reduction of total IgE by targeted coengagement of IgE B-cell receptor and FcγRIIb with fc-engineered antibody. *J Allergy Clin Immunol*. 2012;129:1102-1115.
18. Zhu D, Kepley CL, Zhang M, Zhang K, Saxon A. A novel human immunoglobulin Fcγ-Fcε bifunctional fusion protein inhibits FcεRI-mediated degranulation. *Nat Med*. 2002;8:518-521.
19. Eggel A, Buschor P, Baumann MJ, Amstutz P, Stadler BM, Vogel M. Inhibition of ongoing allergic reactions using a novel anti-IgE DARPin-fc fusion protein. *Allergy*. 2011;66:961-968.
20. Delgado SJ, Dehmel S, Twisterling E, et al. Disruptive anti-IgE inhibitors prevent mast cell–dependent early airway response in viable atopic lung tissue. *J Allergy Clin Immunol*. 2020;145:719-722.e1.
21. Jabs F, Plum M, Laursen NS, et al. Trapping IgE in a closed conformation by mimicking CD23 binding prevents and disrupts FcεRI interaction. *Nat Commun*. 2018;9:7-18.
22. Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C. The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol*. 2005;115:459-465.
23. Peng Z. Vaccines targeting IgE in the treatment of asthma and allergy. *Hum Vaccin*. 2009;5:302-309.
24. Helm B, Kebo D, Vercelli D, et al. Blocking of passive sensitization of human mast cells and basophil granulocytes with IgE antibodies by a recombinant human ε-chain fragment of 76 amino acids. *Proc Natl Acad Sci USA*. 1989;86:9465-9469.
25. Vernersson M, Ledin A, Johansson J, Hellman L. Generation of therapeutic antibody responses against IgE through vaccination. *FASEB J*. 2002;16:875-877.
26. Weeratna RD, Chikh G, Zhang L, et al. Immunogenicity of a peptide-based anti-IgE conjugate vaccine in non-human primates. *Immun Inflamm Dis*. 2016;4:135-147.
27. Engeroff P, Caviezel F, Mueller D, Thoms F, Bachmann MF, Vogel M. CD23 provides a noninflammatory pathway for IgE-allergen complexes. *J Allergy Clin Immunol*. 2020;145:301-311.e4.
28. Engeroff P, Plattner K, Storni F, et al. Glycan-specific IgG anti-IgE autoantibodies are protective against allergic anaphylaxis in a murine model. *J Allergy Clin Immunol*. 2021;147:1430-1441.
29. Zeltins A, West J, Zabel F, El Turabi A, Balke I. Incorporation of tetanus-epitope into virus-like particles achieves vaccine responses even in older recipients in models of psoriasis, Alzheimer's and cat allergy. *NPJ Vaccines*. 2017;2:30.
30. Bachmann MF, Zeltins A, Kalnins G, et al. Vaccination against IL-31 for the treatment of atopic dermatitis in dogs. *J Allergy Clin Immunol*. 2018;142:279-281.e1.
31. Fettelschoss-Gabriel A, Fettelschoss V, Thoms F, et al. Treating insect-bite hypersensitivity in horses with active vaccination against IL-5. *J Allergy Clin Immunol*. 2018;142:1194-1205.e3.
32. Storni F, Zeltins A, Balke I, et al. Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol*. 2020;145:1240-1253.e3.
33. Schmitz N, Dietmeier K, Bauer M, et al. Displaying Fel d1 on virus-like particles prevents reactogenicity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med*. 2009;206:1941-1955.
34. Kaiser L, Grönlund H, Sandalova T, et al. Three-dimensional structure of Fel d 1, the major allergen in cat. *Int Arch Allergy Immunol*. 2003;132:25-26.
35. Thoms F, Jennings GT, Maudrich M, et al. Immunization of cats to induce neutralizing antibodies against Fel d 1, the major feline allergen in human subjects. *J Allergy Clin Immunol*. 2019;144:193-203.
36. Palladino C, Breiteneder H. Peanut allergens. *Mol Immunol*. 2018;100:58-70.
37. Mueller GA, Maleki SJ, Pedersen LC. The molecular basis of peanut allergy topical collection on allergens. *Curr Allergy Asthma Rep*. 2014;14:429.
38. Krenger PS, Sobczak J, Paolucci M, et al. BALB/c and C3H mice are both suitable as peanut allergy models. *Clin Exp Allergy*. 2023;53:1310-1313. doi:10.1111/cea.14398
39. Beck LA, Marcotte GV, MacGlashan D, Togias A, Saini S. Omalizumab-induced reductions in mast cell FcεRI expression and function. *J Allergy Clin Immunol*. 2004;114:527-530.
40. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcεRI expression. *J Allergy Clin Immunol*. 2003;112:1147-1154.
41. Peng Z, Liu Q, Wang Q, Rector E, Ma Y, Warrington R. Novel IgE peptide-based vaccine prevents the increase of IgE and down-regulates elevated IgE in rodents. *Clin Exp Allergy*. 2007;37:1040-1048.
42. Holgate S, Buhl R, Bousquet J, Smith N, Panahloo Z, Jimenez P. The use of omalizumab in the treatment of severe allergic asthma: a clinical experience update. *Respir Med*. 2009;103:1098-1113.
43. Borthakur S, Andrejeva G, McDonnell JM. Basis of the intrinsic flexibility of the Cε3 domain of IgE. *Biochemistry*. 2011;50:4608-4614.
44. Wang CY, Walfield AM, Fang X, et al. Synthetic IgE peptide vaccine for immunotherapy of allergy. *Vaccine*. 2003;21:1580-1590.
45. Chang X, Krenger P, Krueger CC, et al. TLR7 signaling shapes and maintains antibody diversity upon virus-like particle immunization. *Front Immunol*. 2022;12:827256.
46. Gasser P, Tarchevskaya SS, Guntern P, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun*. 2020;11:165.
47. Keeling WF, Martin BJ, Gastrointes BJM. Downloaded from Journals.Physiology.Org/Journal/Jappl at Univ Bern Hosp. 2023.
48. Guntern P, Eggel A. Past, present, and future of anti-IgE biologics. *Allergy*. 2020;75:2491-2502. doi:10.1111/all.14308
49. Drinkwater N, Cossins BP, Keeble AH, et al. Human immunoglobulin E flexes between acutely bent and extended conformations. *Nat Struct Mol Biol*. 2014;21:397-404.
50. Engeroff P, Caviezel F, Storni F, Thoms F, Vogel M, Bachmann MF. Allergens displayed on virus-like particles are highly immunogenic but fail to activate human mast cells. *Allergy*. 2018;73:341-349.
51. Bachmann MF, Rohrer UH, Kündig TM, et al. The influence of antigen organization on B cell responsiveness. *Science*. 1993;262(5138):1448-1451.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Gharailoo Z, Plattner K, Augusto G, Engeroff P, Vogel M, Bachmann MF. Generation of a virus-like particles based vaccine against IgE. *Allergy*. 2024;00:1-15. doi:10.1111/all.16090