Published in final edited form as:

Mol Biosyst. 2013 November ; 9(11): 2853–2859. doi:10.1039/c3mb70286c.

An IgE receptor mimetic peptide (PepE) protects mice from IgE mediated anaphylaxis

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Summary

Crosslinking of receptor-bound Immunoglobulin E (IgE) triggers immediate hypersensitivity reactions including anaphylaxis. Blocking the interaction of IgE with its high-affinity receptor, FcεRI, on mast cells and basophils is an attractive strategy for the treatment of allergies. This approach has seen clinical success using the anti-IgE monoclonal antibody, omalizumab. We recently designed and characterized a novel FcεRI-mimetic peptide (PepE) which contains the two key FcεRI α-chain receptor loops known to interact with the ε-heavy chain of IgE, C'–E and B–C, with an optimized linker for joining them. PepE has high specificity and affinity for IgE, blocks IgE binding to FcεRI and prevents IgE induced mediator release from RBL2H3 cells. We have now investigated the biological effects of this peptide *in vivo* using a line of mice (BALB/c *Il4raF709*) very sensitive to IgE-mediated systemic anaphylaxis. IgE-deficient (IgE^{−/−}) *Il4raF709* mice were passively sensitized with the anti-DNP IgE monoclonal antibody (SPE-7) and subsequently challenged *i.v.* with DNP-BSA. Mice receiving a single dose of PepE prior to sensitization with SPE-7 IgE, were fully protected from anaphylaxis while vehicle control-treated mice displayed strong reactions with significant core body temperature drops and elevated levels of mouse mast cell protease-1 (mMCP-1) in the serum. However, PepE had no effect on IgE-mediated anaphylaxis if given after IgE administration in IgE^{−/−} mice, suggesting that PepE can block binding of free IgE to FcεRI but cannot compete with the receptor for already bound IgE *in vivo*. A single dose of PepE treatment did not protect IgE sufficient mice from IgE mediated anaphylaxis. However, a 3-week long course of PepE treatment protected IgE sufficient *Il4raF709* mice from body temperature drops and elevation of serum mMCP-1. Our findings establish the potential of this type of structure for blocking IgE binding to mast cells *in vivo* and suggest that related peptides might have the potential to attenuate clinical allergic reactions.

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Introduction

Allergic diseases are among the most common chronic disorders in western countries. As much as 22% of the population is affected, establishing allergy as a major healthcare problem. Type I immediate hypersensitivity reactions contribute to the pathogenesis of many allergic diseases including anaphylaxis, drug allergy, food allergy, allergic rhinitis and atopic bronchial asthma^{1–3}. Such hypersensitivity reactions are initiated upon cross-linking of IgE antibodies bound with very high affinity ($K_D = 10^{-9}$ M) to mast cells or basophils via their high affinity receptor, FcεRI. Resultant triggering of the receptor leads to rapid degranulation with release of a variety of preformed chemical mediators, including histamine, and to the biosynthesis of leukotrienes and prostaglandins, which induce vasodilation, increased vascular permeability, up-regulation of vascular adhesion molecules and bronchoconstriction. In the hours following the acute response FcεRI signals also drive a more gradual production of a number of cytokines and chemokines which both drive “late phase” allergic reactions (symptoms occurring 8–12 hours after the initial hypersensitivity response) and orchestrate the inflammatory response of chronic allergic diseases. One of the most dramatic clinical manifestations of immediate hypersensitivity is systemic anaphylaxis. In this syndrome a tremendously bioamplified response to minute amounts of antigen can occur when insect venoms, drugs or foods, interacting with specific IgE antibodies lead to massive mast cell activation. The resultant release of vasoactive mediators leads to vasodilation and plasma extravasation resulting in vascular collapse, shock and occasionally death.

FcεRI, known as the high affinity IgE-receptor, is a multimeric complex including an α-chain that binds Fc-IgE, a β-chain and two linked γ-chains that are essential for cellular signalling³. The binding of IgE-Fc to the α-chain of the FcεRI receptor represents the critical first step in arming mast cells for IgE-mediated activation. Therefore, preventing this interaction would be an effective way to block propagation of signals driving the degranulation response. Molecules capable of selectively blocking IgE binding to FcεRI would have the potential to become a novel and effective class of anti-allergic drugs without the toxicities associated with histamine receptor blockers and glucocorticoids. The clinical success of the anti-IgE monoclonal antibody, omalizumab^{4,5}, has clearly demonstrated the benefits of targeting formation of the IgE-FcεRI complex, and has underscored the potential value of potent and specific small molecule blockers.

Various peptides have been shown to inhibit the IgE-FcεRI interaction. Most of these peptides are linear and were designed based on the structures of IgE and FcεRI^{6–9} or mimicry of Protein A. Others have been derived from bee venom^{10, 11} or identified by screening phage display libraries^{12, 13}. Developing effective antagonists with high affinity and specificity has proven a challenge. Most of those small peptides exhibit good specificity but limited affinity ($IC_{50} > 100\mu M$). Furthermore, most have not been sufficiently characterized in terms of mechanism of binding, and site of recognition. Cell based assays (RBL-2H3 basophilic leukemia cell degranulation mediated by IgE:antigen) as well as *in vivo* approaches, including passive and active cutaneous anaphylaxis have often been used to demonstrate the biological efficacy of those peptides.

We have recently designed and characterized a new FcεRI-mimetic peptide, named PepE, in which the receptor loops C'-E and B-C, corresponding in part to binding site 1 and binding site 2, are joined with an optimized linker^{14, 15}. PepE binds IgE with a unique two-site mechanism and with high selectivity and affinity ($K_D = 500$ nM, comparing to IgE-FcεRIα K_D of 5 nM), also preventing IgE-mediated mediators release from RBL2H3 cells¹⁵.

We have now extended our analysis of the biological functions of PepE to an *in vivo* setting, using a sensitive murine model of systemic anaphylaxis. A passive anaphylaxis protocol, in which *Il4raF709* mice, which express an activated form of IL-4Rα and are exquisitely sensitive to allergic reactions, are sensitized with a monoclonal IgE antibody and then challenged *i.v.* with the target antigen was chosen for these experiments. In order to completely control the IgE environment in the experimental animals, we used IgE deficient (IgE^{-/-}) mice, which completely lack IgE, and gave them SPE-7, a well-characterized DNP-specific IgE monoclonal antibody. Following *i.v.* challenge with polyvalent antigen (DNP-BSA) mast cell degranulation was assessed by measuring serum levels of the granule enzyme, mouse mast cell protease-1 (mMCP-1). In small animals, shock and peripheral vasodilation lead to a fall in core body temperature so this parameter was used as an indicator of the physiologic changes in anaphylaxis¹⁶. We report here that a single dose of PepE pre-treatment fully protected IgE^{-/-} mice from IgE mediated anaphylaxis in a passive anaphylaxis model and that multiple doses of PepE protected IgE sufficient *Il4raF709* mice from body temperature drops and mast cell activation. Our findings show that PepE can block *in vivo* the binding of free IgE to FcεRI, but is unable to displace the immunoglobulins already bound to receptor in the setting of single dose administration. PepE is one of the first compounds with protective effects in a model of systemic anaphylaxis, and as such it has some potential as model for the development of novel anti-allergic drugs.

Materials and Methods

Peptide synthesis and purification

Protected amino acids for the synthesis of peptides were from GL-Biochem (Shanghai, PRC), and Novabiochem (Laufelfingen, Switzerland). The RINK amide resin was from Novabiochem. Coupling agents were from GL-Biochem (Shanghai, PRC); solvents, including acetonitrile (CH₃CN), dimethylformamide (DMF) and methanol (CH₃OH) were from Sigma-Aldrich (Milan, Italy). Other chemicals for the preparation of peptides were also from Sigma-Aldrich (Milan, Italy). An HPLC system from Agilent, equipped with a binary pump, a thermostatic autosampler and a diode array detector was used for the analysis of the peptide in blood samples. Preparative purifications were carried out on a LC8 Shimadzu HPLC system (Shimadzu Corporation, Milan, Italy).

Peptides were synthesized as previously described¹⁵ by the solid phase method following the Fmoc/tBu methodology¹⁷. PepE (See Fig. 1A) was manually prepared on a global scale of 1.0 mmol, whereas the control peptide, designed by scrambling the sequence of the two chains and achieving a molecule with the same MW (See Figure 1B), was prepared on 100 μmoles scale. Canonical Fmoc-protected L-amino acids and RINK amide resin with a 0.57 mmol/g substitution level were used for the synthesis. Boc-Gly-OH was used to introduce the glycine after Y129 for the synthesis of PepE. Most coupling and deprotection reactions

were performed at room temperature for 25 minutes. Fmoc deprotection was achieved by treatment with 30% piperidine in DMF. Couplings with amino acids were performed by activating with 1 equivalent of HATU (2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) and 2 equivalents of DIEA (di-isopropylethylamine). 10 equivalents of Fmoc-protected amino acids were used relative to the synthesis scale. Cleavage of the peptide from the resin was accomplished by treatment with a trifluoroacetic acid (TFA)-triisopropylsilane(TIS)-H₂O mixture (90:5:5, v/v/v) and subsequent precipitation in cold di-ethyl-ether. Peptides were purified by preparative RP-HPLC, using a C18 (5×25cm) 218TP152050 Vydac column operated at a flow rate of 50 mL/min and H₂O and CH₃CN as eluents, both supplemented with 0.05% TFA. Gradients from 10% CH₃CN, 0.05% TFA to 60% in 20 minutes were applied to purify the compounds, monitoring the eluate at 280 nm. About 50 mg of crude material were applied to the column in each run, manually collecting the main peak. Pooled fractions were then lyophilized. Peptides were characterized by LC-MS, determining purity and MW of final compounds (See supplementary Methods). Peptide chiral integrity was assessed by Circular Dichroism (CD) as reported in the Supplementary Methods.

Mice

Animal procedures were performed under protocols approved by the Boston Children's Hospital Institutional Animal Care and Use Committee. IgE deficient (IgE^{-/-}) and IgE sufficient mice on the *Il4ra*^{F709} BALB/c background were described previously^{16, 18}. All mice were bred and maintained under barrier conditions.

Passive anaphylaxis

Six to 8 week old female mice, with body weights of 18 to 20 grams, were treated with a single dose or multiple doses (as indicated in the figure legends) of 2.5 mg of PepE (in 0.5ml PBS with 10% PEG-400, Sigma-Aldrich, 202398, St. Louis, MO.) or an equal volume of vehicle (10% PEG-400 with or without 2.5mg scrambled control peptide or BSA in PBS as indicated) by *i.p.* injection either prior to, or following passive intravenous sensitization by retro-orbital (*r.o.*) injection of 5 µg IgE anti-DNP monoclonal antibody (clone SPE-7, Sigma-Aldrich, St. Louis, MO) in 0.1ml sterile normal saline. PEG-400 was used in the vehicle to improve the PepE solubility. At time points specified in the figure legends, mice were challenged *r.o.* with 500 µg DNP-BSA (Calbiochem, 324101, DNP/albumin ratio 36, EMD Millipore, Billerica, MA) in 0.1 ml sterile normal saline. The doses of IgE and DNP-BSA used in this study were determined in pilot experiments and optimized to achieve significant temperature drops without mortality. All reagents were pre-warmed prior to injection. Core body temperature changes were monitored using an Implantable Programmable Temperature Transponder System (Biomedic Data Systems, Seaford, Del) as previously described¹⁶.

ELISAs

Mouse mast cell protease -1 (mMCP-1), a β-chymase released from mast cell granules during IgE-mediated hypersensitivity responses and mast cell activation⁶, was measured in

serum samples using an ELISA kit from eBioscience (Mouse MCPT-1 Elisa Ready-set-Go, 88-7503, San Diego, CA) according to the manufacturer's instructions.

Pharmacokinetic analysis of PepE

Two mice of each genotype (IgE sufficient and IgE^{-/-}) received 2.5 mg of PepE peptide (0.5ml) dissolved in 10% PEG 400 in PBS via i.p injection at time zero. Blood samples (50 to 100 μ L) were collected into plastic tubes at the following time points: time 0 (prior to PepE i.p. injection), 30 min, 2 hrs, 4 hrs, 6 hrs, 12 hrs, and 24 hrs post the PepE i.p. injection. Serum was collected into EDTA tubes to deactivate proteinases. Serum samples were diluted with distilled water at 4:1 ratio and kept on ice before drying with SpeedVac. Dried samples were stored at -80° C before analysis.

Dried samples were reconstituted by addition of 0.1 M acetic acid to dissociate bound peptide from serum proteins. Then samples were centrifuged for 20 minutes at 12,000 rpm at 4° C. Known volumes (5 or 10 μ L) of these samples were then analyzed by RP-HPLC. Analyses were carried out using a Capcell Pak C18 SG120 column (Shiseido), 5μ m, $4.6\text{mm} \times 250\text{mm}$ and applying a linear gradient of acetonitrile (0.05% TFA) from 25% to 50% over 20 minutes at a flow rate of 0.75 mL/min. Each analysis was repeated three times. A reference curve was obtained by analysing serially diluted calibrated solutions of PepE dissolved in 50% acetonitrile, 0.05% TFA, under the same conditions. Peak areas from chromatograms obtained at 214 nm were integrated and data reported as total amount of peptide in 1 mL of blood sample.

Statistics

Data were analyzed using the GraphPad Prism (version 5.01) software statistics package. The specific tests applied to each data set and the two-tailed *P* values obtained are indicated in figure legends.

Results

Peptide synthesis

PepE (See structure in Figure 1A) was prepared on a global scale of 1.0 mmol. After synthesis and purification, about 0.8 g of pure peptide (>95% by HPLC, see supplementary Figure S1A for analytical characterization)¹⁵ of the final product were obtained, with an overall yield of about 40%. The scrambled control peptide (structure reported in Figure 1B), prepared in a similar way, was obtained with an average yield of 30%, and with a purity of about 95% (See Figure S1B for analytical characterization). Peptide chiral integrity was assessed by CD (Supplementary Figure S2), assessing that for the two peptides, no racemization was achieved during peptide synthesis.

PepE inhibits IgE mediated anaphylaxis in IgE^{-/-} mice

One of the most dramatic manifestations of IgE-mediated hypersensitivity is systemic anaphylaxis. In passive systemic anaphylaxis, an experimental model of this reaction, mice are sensitized to an allergen by administration of specific antibody IgE and then challenged with polyvalent specific antigen. This challenge induces widespread mast cell activation and

degranulation with a resultant increase in plasma levels of the mast cell granule protease, mMCP-1. In addition, the release of vasoactive mast cell mediators leads to vasodilation and increased vascular permeability causing massive plasma extravasation resulting in vascular collapse and shock. Redistribution of blood flow from the central organs to the skin under these conditions leads to cooling so a drop in core body temperature is a sensitive indicator of the physiology of anaphylaxis in mice¹⁶.

We used anaphylaxis-prone BALB/c *Il4raF709* mice to assess the effectiveness of PepE in blocking IgE-mediated mast cell activation *in vivo*. These animals have abundant and sensitive mast cells making them well-suited for studies of anaphylaxis. Normally, IgE antibodies are present in the circulation at physiologic levels that far exceed the K_D of FcεRI. In order to examine the effects of PepE on the initial binding of IgE to its unoccupied receptors *in vivo*, we took advantage of completely IgE-deficient (IgE^{-/-}) mice¹⁸. PepE was given to IgE^{-/-} *Il4raF709* mice *i.p.* 2 hours before the *i.v.* administration of an anti-DNP IgE monoclonal antibody (SPE-7). The animals were challenged with the polyvalent antigen, DNP-BSA, *i.v.* 2 hours after IgE sensitization (four hours after PepE administration). As expected, DNP-BSA challenge of passively sensitized mice resulted in a rapid temperature drop of >3°C, reaching a nadir at 15 minutes (Figure 2A, circles). Pretreatment with a single dose (2.5mg) of PepE significantly dampened the hypothermia induced by DNP-BSA (Figure 2A, squares) ($P < 0.0001$). These animals had a maximum temperature drop of less than 1°C and were already well on the road to recovery at the 15 minute time point when the control animals were displaying their most severe reactions. PepE treatment also led to a reduction in serum levels of the mast cell specific β-chymase, mMCP-1 (Figure 2B), a sensitive indicator of mast cell degranulation ($P < 0.01$). The PepE effect had a relatively short duration. Administration of PepE 8 hours before challenge had no effect on IgE-induced temperature drop or mMCP-1 elevation (data not shown).

PepE does not protect against IgE mediated anaphylaxis when administered after the sensitizing IgE antibody

Our initial observations provided strong evidence that PepE can inhibit IgE mediated reactions *in vivo* when given prior to the sensitizing IgE antibody (Figure 2A and B). In practical clinical application, however, blockers of IgE:FcεRI interactions would be administered in the setting of an established IgE sensitization. Patients have physiologic levels of circulating free IgE antibodies and all of their FcεRI are normally fully saturated. One of the major limitations of the clinically effective IgE blocker, omalizumab, in this context is that it does not disrupt existing interactions between allergen-specific IgE antibodies and FcεRI¹⁹. As a result it must be administered for 6–8 weeks in sensitized individuals, to trap free IgE as well as the IgE dissociated from its receptor, before patients can be safely exposed to allergen²⁰. In order to investigate the efficacy of PepE in preventing allergic reactions in the setting of pre-sensitized mast cells *in vivo*, we passively sensitized a group of IgE^{-/-} mice with SPE-7 prior to PepE treatment. As shown in Figure 3A, giving PepE after IgE sensitization, had no protective effect. PepE- and vehicle control-treated mice had comparable core body temperature drops of about 2.5°C with maximal reactions at 10–15 minutes after challenge. Mean serum mMCP-1 levels were similarly elevated in both groups (Figure 3B). These results indicate that PepE is ineffective in

blocking immediate hypersensitivity reactions mediated by previously sensitized mast cells *in vivo* if only one **single dose** is given to the animals and that it probably has limited ability to compete with FcεRI-bound IgE.

Multiple doses of PepE are required to inhibit IgE mediated anaphylaxis in IgE-sufficient mice

Although IgE is the least abundant circulating immunoglobulin isotype, levels sufficient to saturate FcεRI are present under physiologic conditions in both mice and humans. We hypothesized that the effectiveness of PepE might be altered in the presence of these circulating and FcεRI-bound IgE antibodies. To examine this question we used IgE sufficient *Il4raF709* BALB/c mice. PepE-treated mice exhibited similar core body temperature drops and mMCP-1 elevations as control mice (Figure 4). Taken together, these observations indicate that PepE is ineffective when given as a single dose to animals with normal levels of circulating IgE and saturated IgE receptors. It appears likely that non-specific free IgE was able to bind to and exhaust PepE and decrease the efficacy of PepE binding to specific IgE (anti-DNP IgE, SPE-7).

We observed that mMCP-1 levels did not rise to the same levels in antigen challenged control IgE^{+/+} mice (4,433–9,524 ng/ml, Figures 4B and 5B) as in control IgE^{-/-} animals (37,169–46,272 ng/ml, Figure 2B and 3B). In the IgE^{-/-} mice, all FcεRI would have been unoccupied prior to passive sensitization with SPE-7 IgE MAb allowing all the transferred IgE access to receptor. In contrast, IgE^{+/+} mice receiving IgE presumably had restricted FcεRI availability resulting in a more limited IgE binding to mast cells and then antigen-mediated degranulation but still inducing sufficient mast cell activation to drive the hypothermia response.

To explore the possibility of a protective effect of PepE in wild-type mice subjected to a multiple doses over several weeks (trapping newly-formed IgE antibodies prior to their interaction with FcεRI), we treated a group of IgE sufficient wild-type mice with PepE or control peptide, 2.5mg/mouse/dose, 3 doses per week, for 3 weeks. The animals were sensitized with specific IgE (SPE-7, anti-DNP) 2 hours after the last dose of PepE/control peptide treatment. Challenge was performed with DNP-BSA by *i.v.* injection 2 hours after specific IgE sensitization (Figure 5). Multiple doses of PepE treatment protected wild type mice from IgE mediated anaphylaxis. Animals in the treatment group had reduced temperature drops, with nadirs just over 1 degree, compared with control peptide treated mice which exhibited more than 2 degrees temperature drop.

Pharmacokinetic analysis of PepE

In order to evaluate the pharmacokinetics of PepE following *i.v.* injection, we determined peptide concentrations at 30 min, 2 hrs, 4 hrs, 6 hrs, 12 hrs, and 24 hrs (Figure 6). In both wild type and IgE^{-/-} mice, receiving an initial dose of 2.5 mg PepE, the plasma peptide concentration peaked (between 0.20 and 0.23 mg/mL) after 30 minutes. Assuming a complete distribution of the peptide in the blood stream and an average blood volume of 4 mL, we infer that, even at the peak concentration, only a fraction of the compound initially injected *i.p.*, somewhere between 32% (0.20 mg/mL, 100 μM, IgE^{+/+} mice) and 37% (0.23

mg/mL, 115 μ M, IgE^{-/-} mice), had reached the blood stream. After 2 hrs only about 0.03 mg/mL residual peptide (15 μ M) was present in the blood of IgE^{+/+} mice (0.06 mg/ml, 30 μ M in IgE^{-/-} mice), whereas after 6 hrs, no further compound was detectable indicating a concentration below about 5 μ g/mL (2.5 μ M) given our assay sensitivity. It is notable that all these concentrations are well above the K_D previously established for the IgE:PepE complex (500 nM) suggesting that free IgE should be complexed under these conditions.

Discussion

In recent decades, a number of peptides have been described that inhibit the binding of IgE to its high affinity receptor, Fc ϵ RI⁶⁻¹³. Most of those IgE blockers have been shown to interact with Fc ϵ RI or IgE *in vitro* and RBL-2H3, the rat basophilic leukemia cell line, has been used to demonstrate their functional effects (inhibition of mediator release). The effectiveness of these compounds *in vivo* has been assayed using passive *cutaneous* anaphylaxis, a model of dermal mast cell activation. We have previously reported that the Fc ϵ RI-mimetic peptide (PepE), which contains the two key Fc ϵ RI α -chain receptor loops known to interact with the ϵ -heavy chain of IgE, C'-E and B-C, with an optimized linker for joining them, inhibits IgE mediated RBL-2H3 cell degranulation¹⁵. We now demonstrate the effectiveness of PepE *in vivo*. We use the novel approach of passive *systemic* anaphylaxis which has the benefits of providing quantitative read-outs (core body temperature and mMCP-1 levels) and reflecting a relevant clinical scenario of intense IgE mediated hypersensitivity.

In our study, a single dose of PepE completely blocked antigen:IgE mediated systemic anaphylaxis in IgE^{-/-} mice, inhibiting hypothermia, an indicator of shock, and mMCP-1 release, a measure of systemic mast cell degranulation. This result clearly demonstrates the efficacy of PepE *in vivo*. However, the protective effect was short lived. Mice treated with PepE 4 hours before challenge exhibited no response to IgE:antigen but those receiving the same dose of inhibitor 8 hours prior to antigen challenge were not protected from anaphylaxis. Furthermore, a single dose of PepE did not confer any protection to pre-sensitized animals (Figure 3). This was an anticipated result since PepE, as an Fc ϵ RI mimetic peptide interacting with the same Fc ϵ sites as Fc ϵ RI, would have no ability to bind IgE already complexed with Fc ϵ RI. This finding confirms that while PepE has a high affinity for IgE *in vitro*¹⁵, it may not be able to displace Fc ϵ RI-bound IgE *in vivo*. Under these circumstances, a single dose of PepE injection would be unlikely to change the fate of IgE mediated responses in pre-sensitized mice in which mast cell and basophil Fc ϵ RI is fully occupied by specific IgE. This situation is analogous to that observed with omalizumab (humanized anti-human IgE) which also interacts with Fc ϵ RI-interacting domains of Fc ϵ and has no ability to displace receptor-bound IgE.

Similarly, a single dose PepE did not show any protection against IgE mediated anaphylaxis in IgE sufficient *Il4raF709*/BALB/c mice (Figure 4). This result is likely related to the saturation of injected PepE by nonspecific background IgE in wild type mice leaving insufficient free PepE to block the additional passively administered antigen-specific IgE (SPE-7). In contrast multiple doses of PepE treatments over several weeks provided protection even in IgE-sufficient animals (Figure 5). Clinical experience with omalizumab

similarly suggests that several weeks of pre-dosing is effective in blocking IgE-mediated immediate hypersensitivity reactions^{4,20}. This positive result suggests a potential strategy for peptide-based IgE blockade in IgE-sufficient subjects.

Using a quantitative and sensitive animal model for antibody:antigen-induced anaphylaxis, we have demonstrated that the FcεRI mimetic peptide, PepE can completely block IgE:FcεRI mediated anaphylaxis when given prior to IgE sensitization. We believe this report validates the concept that further development of novel FcεRI-mimetic peptides like PepE will lead to potentially-useful compounds for the treatment of allergic diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the support from Regione Campania for the Project “Rete di Eccellenza Strategie Terapeutiche Innovative” (STRAIN) for a fellowship to V.S. and Progetto CAMPUS; from CNR (Consiglio Nazionale delle Ricerche) for the project “Collezione di Composti Chimici ed attività di screening”, and MIUR for the project “FIRB-MERIT N° RBNE08NKH7” to Dr. Menotti Ruvo. Dr. Joseph Zhou is supported by a fellowship from NIH NIAID (T32AI007512). Dr. Hans Oettgen is supported by an NIH/NIAID grant, R56 AI100889-01.

Abbreviations

DMF	Dimethylformamide
DCM	Dichloromethane
DIEA	Di-isopropylethylamine
Fmoc	Fluorenylmethoxycarbonyl
HBTU	1-H-Benzotriazolium, 1-[bis(dimethylamino)methylene]-hexafluorophosphate(1-),3-oxide
HOBt	N-hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
TIS	Tri-isopropylsilane
TFA	Trifluoroacetic acid

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Figure 1. Single-letter code representation of peptides used in this study

In A the IgE-binding peptide PepE is sketched. The peptide was prepared as described in reference 15, starting from the C-terminal H134. A β -alanine (β A), was introduced on the side chain of the lysine to make the linker longer and more flexible. In B, the primary structure of the scrambled peptide used as negative control is reported. The peptide was acetylated on the N-terminus of tryptophan on the first chain.

Figure 2. PepE inhibits IgE mediated anaphylaxis in IL4raF709 IgE^{-/-} mice

Six to 8 weeks old Female *IL4raF709* IgE^{-/-} mice with body weights of 18 to 20 grams treated with 2.5mg PepE (n=4) or vehicle (0.5ml of 10% PEG in PBS, via *i.p.* injection) (n=6) at -4hrs were sensitized with 5μg of SPE-7 IgE (in 0.1ml sterile saline, via *r.o.* injection) at -2hrs and then challenged with 500μg of DNP-BSA (in 0.1ml sterile saline via *r.o.* injection) at time 0. **A.** Core body temperature at different time points (mean ± SEM) after DNP-BSA challenge (AVOVA, p<0.0001). **B.** Serum mMCP-1 levels. Each point represents one individual mouse; the thin longer horizon line and two thicker shorter horizon lines represent the group mean and standard error respectively. mMCP-1 levels from 3 naïve mice were presented as reference. Mean ± SEM mMCP-1: 46,272 ± 9,383 in the control group, 7,140 ± 630 in the PepE group and 77.3 ± 6.1 in the naïve group of mice after DNP-BSA challenge (ANOVA, P<0.01). The data presented are from a single experiment and are representative of the results of three independent replicate experiments.

Figure 3. PepE does not confer protection against IgE mediated anaphylaxis in previously sensitized animals

Female *Il4raF709* IgE^{-/-} mice with body weights of 18 to 20 grams sensitized with IgE (SPE-7) (in 0.1ml sterile saline, via *r.o.* injection) at -4hrs were treated with PepE (n=5) or vehicle PEG (in 0.5ml of 10% PEG in PBS, via *i.p.* injection)(n=5) at -2hrs and then challenged with DNP-BSA (in 0.1ml sterile saline via *r.o.* injection) at time 0. **A.** Core body temperature (mean ± SEM) after DNP-BSA challenge. **B.** Serum mMCP-1 levels. Each dot represents the value of an individual mouse; the thin longer horizon line and two thicker shorter horizon lines represent the group mean and standard error respectively. Mean ± SEM mMCP-1: 37,169 ± 10,172 in the control group, 32,437 ± 6105 in the PepE group and 77.3 ± 6.1 in the Naive group of mice after DNP-BSA challenge (ANOVA, P>0.05). mMCP-1 levels from 3 naïve mice are presented for reference. The data presented are from a single experiment and are representative of the results of three independent repeat experiments.

Figure 4. PepE does not inhibit IgE mediated anaphylaxis in IgE-sufficient mice
 IgE^{+/+}*Il4ra*^{F709} BALB/c mice with body weight of 18 to 20grams were treated with PepE (n=4) or vehicle (n=4) (2.5mg BSA in 0.5ml of 10% PEG, via i.p. injection) at -4hrs and SPE-7 IgE (in 0.1ml sterile saline, via *r.o.* injection) at -2hrs. The mice were challenged with DNP-BSA (in 0.1ml sterile saline via *r.o.* injection) at time 0. **A.** Core body temperature at different time points (mean ± SEM) after DNP-BSA challenge (ANOVA, $P>0.05$). **B.** Serum mMCP-1 levels of mice. Each dot represents the value of an individual mouse; the thin longer horizon line and two thicker shorter horizon lines represent the group mean and standard error respectively. Mean ± SEM mMCP-1: 4,433 ± 2,579 in the control group and 3,375 ± 1,937 in the PepE group after DNP-BSA challenge (T-test, $P>0.05$). The data presented are from a single experiment and are representative of the results of three independent repeat experiments.

Figure 5. Multiple doses of PepE treatment protected IgE^{+/+} IL4raF709 mice from systemic anaphylaxis

Six week-old female IgE sufficient *Il4raF709* BALB/c mice (n=5 in each group) were treated with PepE or control peptide by *i.p.* injection (2.5 mg in 0.5ml 10% PEG-400), 3 times a week for 3 weeks. All animals were then sensitized with SPE-7 anti-DNP IgE (5 μ g in 0.1ml sterile saline) 2 hours after the last dose of PepE or control peptide treatment. They were then challenged with DNP-BSA (500 μ g in 0.1ml sterile saline) 2 hours after IgE sensitization. A. Core body temperature at different time points (mean $\pm$ SEM) after DNP-BSA challenge (ANOVA, $P < 0.0001$). B. Serum mMCP-1 levels of mice. Each dot represents the value of an individual mouse; the thin longer horizon line and two thicker shorter horizon lines represent the group mean and standard error respectively. Mean $\pm$ SEM mMCP-1: $9,524 \pm 5,610$ in the control group and $1,330 \pm 524$ in the PepE group after DNP-BSA challenge.

Figure 6. PepE pharmacokinetics

Female wild type IgE sufficient and IgE^{-/-} BALB/c mice with body weight of 18 to 20grams (n=2 in each group) received 2.5mg PepE (in 0.5ml of 10% PEG, via i.p. injection) after time 0 blood collection. Blood samples were then collected at 30min, 2hrs, 4hrs, 6hrs, 12hrs, and 24hrs. Serum PepE levels were determined by HPLC. **A.** Wild type BALB/c mice; **B.** IgE^{-/-} mice. HPLC analyses were repeated 3 times for every serum sample; each data point represents the mean ± SEM (six data points from the two animals). The serum levels of PepE at 12hrs and 24hrs were undetectable and were not reported in the graph.