

Cytotoxic T-cells specific for natural IgE peptides downregulate IgE production

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In dedication to Dr. Zoltan Ovary on the 48th anniversary of discovery of PCA reaction

Abstract

Immunoglobulin E (IgE) plays a central role in IgE-mediated immediate type hypersensitivity. Since production of IgE depends on Th2, efforts to block IgE production and control allergic reactions include tolerization of Th2 or deviating development of Th2. We hypothesized that cytotoxic T lymphocytes targeting natural IgE peptides/MHC I complexes can eliminate IgE-producing cells and inhibit centrally IgE production. CTL to self-IgE peptides were elicited in mice immunized with nonameric p^{109–117}, p^{113–121}, and p^{103–141} (CHε2 domain), which encompass both peptides with an OVA helper peptide (OVAp restricted for H-2^{d/b}) in liposomes and presented by dendritic cells (DC). CTL from BALB/c lysed IgE peptide-pulsed P815 target as well as IgE-producing 26.82 hybridomas (H-2^d). Natural tolerance to self-IgE peptides was tested in IgE sufficient (IgE +/+) as well as IgE-deficient (IgE –/–) 129/SvEv mice (H-2^b). Comparable magnitude of CTL responses was observed in both strains immunized with p^{109–117} or p^{103–141} concomitantly with CD4 T-cell costimulation. CTL from 129/SvEv lysed not only IgE peptide-pulsed EL-4 but also IgE-producing B4 hybridomas (H-2^b). This observation strongly suggests a correspondence of epitope of immunogenic peptide to that of physiologically processed IgE peptides presented on IgE-producing cells. Moreover, CTL were generated in 129/SvEv, immunized with the recombinant antigenized antibody in liposomes encompassing p^{107–123}, p^{109–117}, and p^{113–121} expressed in CDR3 of VH62/human γ1. Polyclonal IgE production was inhibited by coinubation with MHC I-restricted CTL in vitro. Furthermore, antigen-specific IgE responses were inhibited in mice, immunized with p^{109–117} and p^{103–141} while IgG responses were not suppressed. Since IgE peptide sequences of CHε2 are ubiquitous to all murine IgE heavy chain, peptides made as such can serve as a universal IgE vaccine to prevent allergy for a myriad of allergens in rodents. This observation suggests that similar human IgE peptides should be identified and employed to downregulate human IgE production.

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1. Introduction

Immunoglobulin E (IgE)¹ plays a central role in immediate type hypersensitivity such as allergic asthma,

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¹ Abbreviations used: CTL, cytotoxic T lymphocytes; IgE, immunoglobulin E; CHε2, constant heavy chain epsilon 2; CDR3, complementarity-determining region 3; DC, dendritic cells; DOTAP, a cationic liposome; GM-CSF, granulocyte-macrophage-colony stimulating factor; IL-4, interleukin 4; IM, internal image; MAb-E25, monoclonal antibody-E25; Th2, T helper cell subset 2.

rhinitis, food allergy, atopic dermatitis, and anaphylaxis [1,2]. IgE-mediated allergic inflammation can be controlled at two levels: by antagonizing the pharmacological effect of inflammatory mediators and cytokines [3,4], or by inhibiting the production of allergen-specific IgE [5–7]. IL-4, IL-13, and CD40 play a crucial role on IgE B-cell commitment via their induction of sterile germ-line IgE heavy chain gene transcript as a prerequisite for proper VDJ recombination with the CH ϵ gene. IFN- γ on the other hand inhibits IgE germ-line gene transcript [8–11]. Complex regulation of IgE production is also at the level of Th1/Th2 dichotomy with IL-4 and IL-12/IFN- γ playing opposing roles [12,13]. Downregulating IL-4 production thus attenuates IgE production by affecting both IgE class switch and Th2 development. Conventional immunotherapy requires that pertinent CD4 helper T-cell epitopes of the respective allergen be identified, and immune deviation be appropriately implemented [14]. However, variable and paradoxical results are often obtained when allergen helper peptide epitopes were employed for desensitization and immune deviation [14,15].

Despite the complexity of controlling IgE B-cell differentiation, downregulating IgE levels can be achieved by IgE immunization, bypassing the cytokine regulatory circuits. Previously, our laboratory observed

p^{113–121}, costimulated with OVA helper peptide (OVAp) in liposomes and/or presented by dendritic cells (DC). IgE peptide-specific CTL lyse peptide-pulsed conventional CTL targets as well as IgE-producing cells. Antigen-specific IgE and total serum IgE levels in vivo were concomitantly reduced in IgE peptide-immunized mice. We propose that cytotoxic IgE peptide epitope(s), derived from CH ϵ 2 to CH ϵ 4 domain, may be employed as a universal allergy vaccine. CTL elicited by the vaccines recognize natural IgE peptides/MHC class I on IgE-producing cells, and therefore eliminate/inhibit IgE production irrespective of allergen specificities [16].

2. Materials and methods

2.1. Antigen

IgE peptides LLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDRE (p^{103–141}); LYCFIYGHILNDV (p^{109–117}, restricted to both K^d and K^b); and IYGHILNDV (p^{113–121} nonamer, restricted to D^d). Numbering the amino acid followed the convention of Liu et al. [21], with the beginning amino acid of first constant region, designated as number 1, and residue 103

LLHSSCDPNAFHSTIQLYCFIYGHILNDVSVSWLMDDRE 39mer peptide (p^{103–141})
 LYCFIYGHILNDV 9-mer peptide H-2K^d, H-2K^b (p^{109–117})
 IYGHILNDV 9-mer peptide H-2D^d (p^{113–121})

that active IgE immunization contributed to diminished IgE production in a murine model [17–20]. Both cell-mediated immunity and humoral anti-IgE antibodies play a role in suppressing IgE responses of IgE-immunized hosts. Monoclonal anti-IgE prevents IgE binding to high affinity IgE receptors on mast cells without activating IgE-bound mast cells. Omalizumab (Xolair) introduced into the market based on this principle effectively neutralize circulating IgE and relieve syndromes in patients with severe allergic asthma [46–48].

The role of cell-mediated immunity, in addition to humoral anti-IgE, in this perinatal IgE immunization model was studied in this laboratory. CD8(+) T cells were identified to play a crucial role in the induction and maintenance of total IgE deficiency as shown by the adoptive transfer study in vivo as well as by cocubation of CD8(+) T cells and IgE-producing cells in vitro. Peptide I, a 39-mer fragment (p^{103–141}), sequenced and mapped in the CH ϵ 2 exhibited profound IgE deficiency [16–20]. Herein, we hypothesize that CTL from adult mice immunized with p^{103–141} and its resident MHC I-binding nonameric peptides can lyse IgE-producing cells as target, and thereby inhibit de novo IgE synthesis. We report that CTL tolerance to self-IgE peptides was broken in immunocompetent adult mice, with p^{103–141}, nonameric p^{109–117}, and

starting as the third amino acid residue from CH ϵ 2 [16,21]. ISQAVHAAHAEINEAGR (OVAp^{323–339}, restricted to both IA^d and IA^b) [22] were synthesized in the peptide facility of La Jolla Institute for Allergy and Immunology, San Diego, CA. Peptides are purified by HPLC with more than 95% purity and M.W. ascertained by time-of flight of mass spectrometry. Recombinant murine GM-CSF was obtained from Kirin Pharmaceutical Lab or Biosource International (Cambridge, MA). Cationic liposome, N-[1-(-2,3-dioleoyloxy)propyl]-N,N,N-trimethylammoniummethylsulfate (DOTAP), was purchased from Boehringer–Mannheim (Indianapolis, IN). Rat anti-CD40 (FGK-45) was a gift from Dr. Stephen Schoenberger (LIAI, San Diego, CA). A pair of oligonucleotides corresponding to the sense and anti-sense strands of the overlapping 17-mer p^{107–123} cytotoxic epitopes (IQLYCFIYGHILNDVSV) were synthesized: 5'-GTACCCATCCAGCTGTACTGCTTCATTTATGGCCACATCCTAAATGATGTCTCTGTC-3' (sense strand); GTACGACAGAGACATCATTTAGGATGTGGCCATAAATGAAGCAGTACAGCTGGATGG-3' (anti-sense), cloned or antigenized into the Asp 716 (*Kpn*I isochizomer) restriction site of the murine VH62 fused to Fc of human IgG1 with the neomycin selection vector as previously reported [23,24].

2.2. Dendritic cell cultures

Bone marrow-derived dendritic cells (BMDC) were prepared from bone marrow of 6-week-old female BALB/c or 129/SvEv by culturing femur bone marrow cells in the presence of 1.5 ng/ml recombinant GM-CSF over a period of 10 days following a decanting procedure described by Inaba and colleagues [25]. The purity of DC (>90%) was determined by MAb against CD11c (integrin α_x chain, Pharmingen). 1×10^6 DC were pulsed with $p^{103-141}$ + OVAp at a final concentration of 100 μ g/ml, entrapped in DOTAP liposomes at a final concentration of 30 μ g/ml for 1 h at 37°C. Unbound peptides and liposomes were removed by washing.

2.3. Immunization and assays

Female 8-week-old BALB/c, 129/SvEv wild-type mice were obtained from Jackson and Tacomc Laboratories, respectively. IgE gene-deficient mice (129/SvEv, IgE $-/-$) mice were kindly obtained from Dr. Hans Oettgen [26] (Harvard University, Boston, MA). IgE peptides and OVAp helper peptides were added to DOTAP liposomes at equal volumes, and incubated at r.t. for 1 h. Each mouse was then immunized twice with 30 μ g IgE peptide and 30 μ g OVAp helper peptide, mixed in 30 μ g of liposomes divided equally in two separate routes via i.p. and the base of tail at an interval of 10–14 days. Each mouse in a group of four to six was injected with 2×10^4 peptide-pulsed DC in toto with 0.15 ml injected s.c., and 0.15 ml injected i.p. Mice were then boosted with the corresponding antigen formulation. One hundred micrograms of anti-CD40 antibody (FGK-45) was injected i.p. 1 day after peptide immunization. Passive cutaneous anaphylaxis (PCA), total IgE measurement, and antigen-specific IgG assays were described previously [19,20,27].

2.4. RMA-S MHC I upregulation assay

RMA-S (obtained from Dr. Steve Schoenberger) grown at 37°C exhibited temperature-sensitive TAP-1 that failed to stably express surface MHC I. Cells were incubated in the presence of nonameric IgE peptide, $p^{109-117}$ or with positive control S9 Kb/Db binder peptide (FAPGNYPAL) [28] at 10–100 μ g/ml for 8 h at 25°C and shifted to 37°C overnight. Quantitative surface expression of D^b and K^b was detected by FITC conjugates of anti-D^b (clone 28.14.8S) and anti-K^b (clone B8.3.24), respectively.

2.5. CTL assay

Fourteen to 21 days after antigenic boosting, 5×10^6 ml spleen cells from at least two mice were re-stimulated in vitro with 50 μ g/ml IgE peptide and 50 μ g/ml OVAp in 24-well for 6–7 days. 5×10^6 P815 or EL-4 were incubated with 50 μ g $p^{103-141}$ /ml in the presence of 50 U IFN- γ /ml

overnight, or pulsed for 2 h with 50 μ g/ml nonameric IgE peptides ($p^{109-117}$, $p^{113-121}$) in the absence of IFN- γ . Target cells were then added with 0.6 mCi ^{51} Cr for 1–2 h at 37°C. To prepare IgE-secreting cells as direct targets, 26.82 (DNP-specific IgE-secreting hybridoma, H-2^d) [29] and B4 (TNP-specific IgE-secreting hybridoma, H-2^b) [30] were incubated with ^{51} Cr directly. In vitro re-stimulated spleen cells were then added to 1×10^4 ^{51} Cr-labeled target cells at E/T ratios from 100:1 to 1:1 for 4–6 h. The specific lysis was determined by the following formula: (experimental release – spontaneous release/maximal release – spontaneous release) $\times$ 100. Maximal release was performed by evaluating ^{51} Cr in the supernatant by PBS with 1% Triton. Preparation and separation of subsets of CD4 versus CD8 were performed as described [19,20].

3. Results

3.1. Two signals are required for breaking tolerance to self-IgE peptides

Peptide I, $p^{103-141}$ previously mapped by V8 protease was known to cause IgE deficiency in perinatally injected mice prior to the onset of self-tolerance [16]. To determine whether CTL may be induced in adult mice, mice were immunized with $p^{103-141}$ in the presence of CD4 costimulation. Fig. 1A showed that this self-IgE peptide fragment alone did not induce CTL responses in adult mice, and was rendered immunogenic with CD4 T-cell costimulation [31,32]. Thus, CTL were not elicited with injection of $p^{103-141}$ alone, or costimulated with OVAp in liposomes. In contrast, CTL elicited in mice immunized with $p^{103-141}$ and OVAp helper peptide in saline or in liposomes lysed $p^{103-141}$ and IFN- γ -treated P815 targets (44–56% chromium release at E/T 100:1).

To determine whether immunogenicity of $p^{103-141}$ was due to H-2K^d/H-2D^d nonameric binder motifs within $p^{103-141}$, mice were immunized with nonameric $p^{109-117}$, and $p^{131-121}$ along with OVAp in liposomes. Fig. 1B showed that CTL from $p^{109-117}$ immunized mice caused more efficient chromium release than those from $p^{103-141}$ ($p < 0.05$), and $p^{113-121}$ -immunized mice ($p < 0.01$), i.e., 48% release versus that of 22%. This observation suggests that $p^{109-117}$ and $p^{113-121}$ are the active immunogenic principles of $p^{103-141}$. Fig. 1C showed that augmented cell-mediated cytotoxicity was observed in CD8-enriched population as compared to un-fractionated spleen cells. Anti-CD8 treatment abrogated cytotoxic activities of $p^{103-141}$ -restimulated cultures of spleen cells.

3.2. CTL induced by IgE peptide-pulsed DC lyse IgE-producing hybridomas

The trans-migratory property of DC is a desirable property as a vehicle for eliciting protective immunity

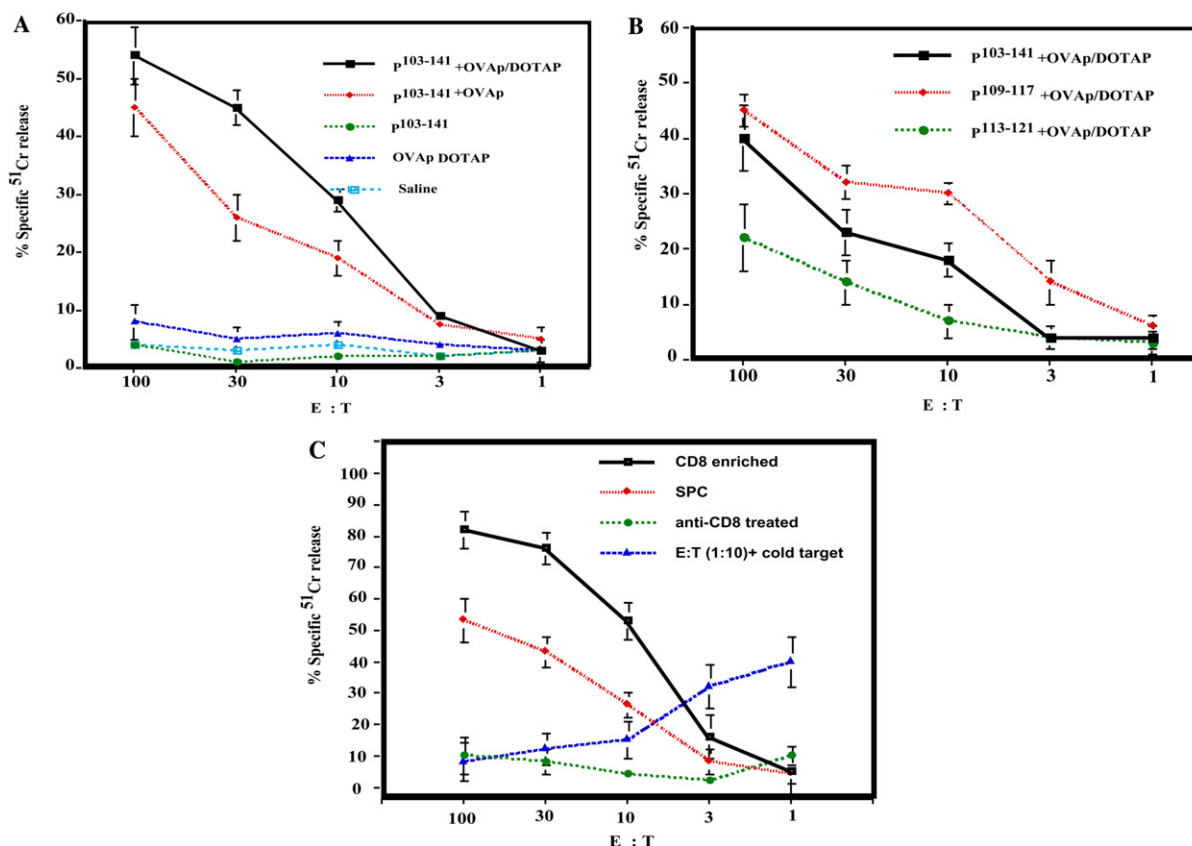


Fig. 1. Induction of IgE-specific CTL by IgE peptides/liposome complexes. Eight-week-old BALB/c mice were primed with 30 μg p¹⁰³⁻¹⁴¹ alone (LLHSSCDPNAFHSTIQLYCFIYGHLNDVSVSWLMDDRE), or 30 μg of the helper peptide, OVAp (ISQAVHAHAHAEINEAGR) in 30 μg DOTAP, a cationic liposome preparation. Two weeks later, mice in each respective group were challenged with the corresponding antigen used in the initial priming. Two weeks after the second immunization, 5×10^6 spleen cells from each group were established in vitro, and re-stimulated with antigen in vitro for 7 days. P815 as targets were pulsed with p¹⁰³⁻¹⁴¹, and treated overnight with 50 U/ml IFN- γ . Effector and targets were mixed at different E/T ratios for 4 h. Specific lysis was calculated as specified above (A). Mice are immunized with the two respective nonameric peptides, 30 μg p¹⁰⁹⁻¹¹⁷ (LYCFIYGHI) and p¹¹³⁻¹²¹ (IYGHLNDV) exhibiting the respective cytotoxic T-cell motifs along with 30 μg OVAp helper peptides in liposomes. P815 targets were directly pulsed with 30 μg of nonameric peptides for 1 h. CTL peptides were further processed and presented by; and the antigenic peptide counterpart may also be processed by the P815 due to overnight IFN- γ incubation that enhances immunoproteasome activities [40] (B). Splenocytes from p¹⁰³⁻¹⁴¹-immunized mice were depleted with anti-CD8 plus complement or enriched by anti-CD8 column on magnetic beads. p¹⁰³⁻¹⁴¹-pulsed cold targets were added to E:T cultures with the ratios of cold versus hot targets from 1 to 100 (C).

extended in different lymphoid compartments [33]. We therefore determine whether self-IgE peptides and OVAp pulsed onto dendritic cells (DC) may break tolerance to self-IgE, and whether anti-CD40 (FGK-45) modulated CTL induction [34,35]. Adult BALB/c mice were treated with the anti-CD40 (FGK-45) i.p. 24 h after immunization with IgE p¹⁰³⁻¹⁴¹ + OVAp-pulsed DC. Fig. 2A showed that p¹⁰³⁻¹⁴¹ and OVAp presented by DC elicited IgE peptide-specific CTL that lysed p¹⁰³⁻¹⁴¹-pulsed P815 targets. Interestingly, in vivo anti-CD40 treatment with IgE peptide-pulsed DC with or without liposomes did not further augment, and on the contrary downregulated CTL induction.

We wish to determine whether IgE peptide-specific CTL target IgE-producing cells, and therefore will be relevant in downregulating pathophysiological levels of IgE. IgE-secreting hybridomas 26.82 (H-2^d) [29] were employed as a natural target, since it expressed high

levels of IgE molecules. Fig. 2B showed that CTL-induced peptide-pulsed DC lysed also 26.82 IgE hybridomas. To determine the immunogenicity of nonameric p¹⁰⁹⁻¹¹⁷, BALB/c mice were immunized with p¹⁰⁹⁻¹¹⁷ and OVAp pulsed DC, Fig. 2C showed that CTL induced in BALB/c mice lysed p¹⁰⁹⁻¹¹⁷-pulsed P815 and 26.82 IgE-secreting hybridomas (H-2d). To extend this observation to IgE-producing hybridoma of a different haplotype, DC were similarly prepared in 129/SvEv mice (H-2^b). 129SvEv were immunized with DC pulsed with p¹⁰⁹⁻¹¹⁷ (with dual H-2^{d/b}-binding nonameric motif) and OVAp. Fig. 2D showed that CTL induced in this H-2^b mice lysed p¹⁰⁹⁻¹¹⁷-pulsed EL4 as well as another high rate of IgE-secreting hybridomas (B4 of the H-2^b haplotype) [30] with comparable efficacies. MHC class I restriction holds for the p¹⁰⁹⁻¹¹⁷ peptide with respect to the H-2^d versus H-2^b haplotype. Thus, p¹⁰⁹⁻¹¹⁷/H-2d-restricted CTL lysed IgE-producing 26.82 (H-2^d) but not IgE producing B4 (H-2^b).

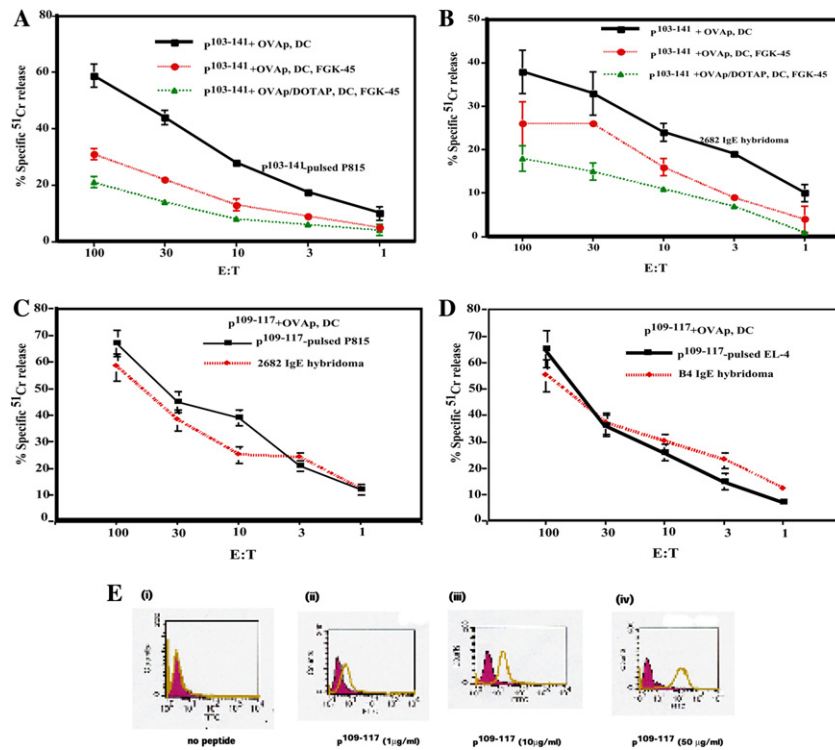


Fig. 2. CTL induced by IgE peptide-pulsed dendritic cells lyse IgE-secreting hybridomas. Bone marrow-derived dendritic cells (BMDC) were prepared by culturing femur bone marrow cells of 6-week-old BALB/c or 129/SvEv mice in the presence of 1.5 ng/ml murine recombinant GM-CSF for 10 days [27]. 1×10^6 DC were pulsed with 100 μg p¹⁰³⁻¹⁴¹ or p¹⁰⁹⁻¹¹⁷ + OVAp in liposomes for 1 h at 37 °C. Unbound peptides were washed and each mouse in a group of four was injected s.c./i.p., with 2×10^4 peptide-pulsed DC into BALB/c and 129/SvEv, respectively. Mice were boosted likewise 2 weeks later, and the spleen cells were re-stimulated in vitro with p¹⁰³⁻¹⁴¹ or p¹⁰⁹⁻¹¹⁷ + OVAp for 7 days, respectively. BALB/c effectors were added to p¹⁰³⁻¹⁴¹-pulsed P815 as targets (A), to 2682 IgE-secreting hybridomas (B), and to p¹⁰⁹⁻¹¹⁷-pulsed P815 and 2682 (C). 129/SvEv effectors were added to p¹⁰⁹⁻¹¹⁷-pulsed EL-4 and B4 targets (D). RMA-S-A2Kb was incubated with p¹⁰⁹⁻¹¹⁷ at 1, 10, and 50 $\mu\text{g}/\text{ml}$ (ii–iv) overnight and cell surface expression of HLA-A2Kb was detected by FITC-anti-HLA-A2.1 (E).

Conversely, p¹⁰⁹⁻¹¹⁷-specific CTL lyse IgE producing B4 hybridoma but not IgE producing 2682 (H-2^b) (not shown).

The suitability of IgE-secreting plasma cells as targets is concordant with the observation that peptide-pulsed activated B cells are routinely employed as stimulators for CTL in a typical chromium release assay [36,39]. The above observations strongly suggest the correspondence of synthetic peptides employed herein to the physiologically processed IgE peptides presented by MHC I by IgE-producing cells as CTL targets.

To formally determine that physical binding of p¹⁰⁹⁻¹¹⁷ to MHC I, we proceeded to determine whether p¹⁰⁹⁻¹¹⁷ can bind and cause stable surface expression of MHC class I in TAP-1-deficient RMA-S cell line [28]. As shown in Fig. 3E, H-2K^b was not detectable in mutant RMA-S incubated in normal media first in 25 °C and shifted to 37 °C overnight (panel i). Elevated levels of H-2K^b were stably expressed in mutant cells, incubated in 25 °C in the presence of p¹⁰⁹⁻¹¹⁷ at 1–100 $\mu\text{g}/\text{ml}$, then shifted to 37 °C (panels ii–iv, respectively). Mutant RMA-S incubated with S9 positive control peptide at 100 $\mu\text{g}/\text{ml}$ expressed high levels of H-2K^b (not shown).

3.3. Comparable magnitude of MHC I-restricted, IgE peptide-specific CTL induced in IgE deficient and wild mice

To determine whether breaking tolerance to self-IgE was complete, the magnitude of CTL responses induced in wild-type mice (129 SvEv (H-2^b), IgE +/+) was then compared to that of IgE gene-deficient mice (129/SvEv, IgE –/–) [26], and tolerance of CD8 T cells to IgE did not develop since self-IgE is not present. It is noted that p¹⁰³⁻¹⁴¹ exhibits motif for nonameric K^b binder, p¹⁰⁹⁻¹¹⁷. Therefore was delivered to IgE wild-type (129, IgE +/+) and IgE-knock-out mice (129, IgE –/–) with different costimulation.

Herein, the results showed three pertinent points. First, Fig. 3A showed that immunization with p¹⁰³⁻¹⁴¹ + OVAp-pulsed DC, or p¹⁰³⁻¹⁴¹ + OVAp in liposomes elicited CTL in 129 IgE –/– mice which lyse IgE-producing B4 hybridomas [30] (38–47% lysis at E/T 100:1). This suggests that the natural peptide sequence processed and presented by DC is similar to the processed IgE peptide, presented by MHC I in IgE-secreting plasma cells. CTL lysed B4 IgE-producing cells and peptide-pulsed EL-4 with comparable efficiencies (46–58%

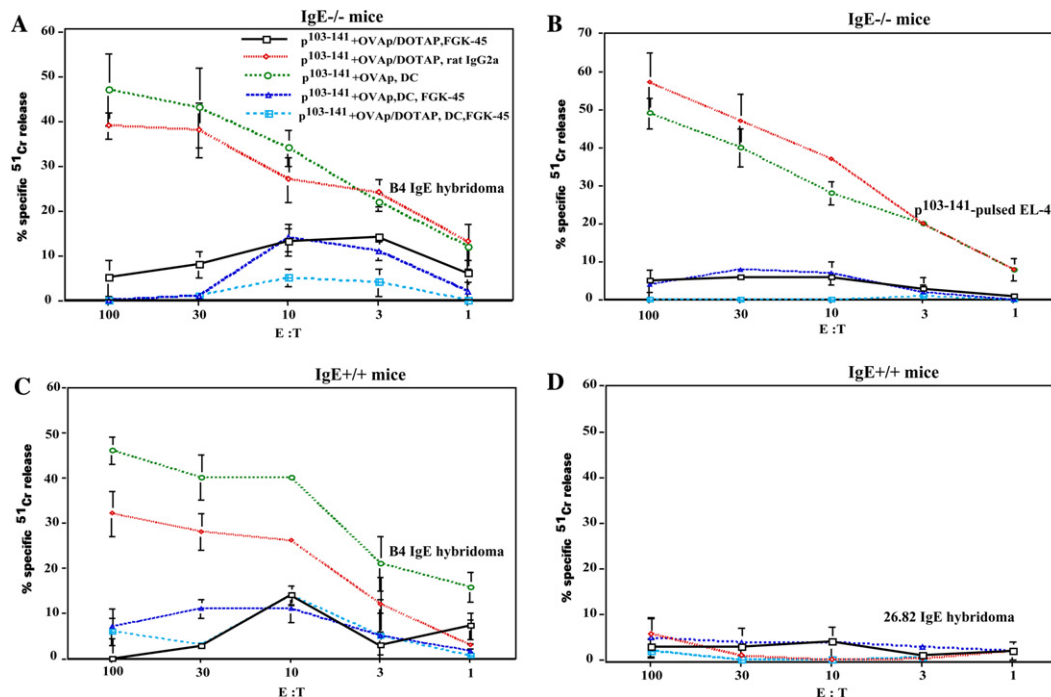


Fig. 3. Induction of IgE-specific CTL in both IgE-tolerant and non-tolerant mice. IgE gene-deficient mice, i.e., 129/SvEv, IgE $-/-$ and 129/SvEv, IgE $+/+$ wild-type mice were immunized with $p^{103-141}$, of which the delimited, synthetic $p^{109-117}$ binder translocated K^b on cell surface of TAP2($-/-$) RMA-S mutants. Mice were immunized with $30 \mu\text{g } p^{103-141}$, $30 \mu\text{g OVAp}$ in $30 \mu\text{g}$ liposomes in a total of $150 \mu\text{l}$, injected equally via the s.c./i.p. routes. Twenty-four hours after injection, mice were further injected i.v. with FGK-45 (anti-CD40) or normal rat IgG as control. Moreover, mice were first immunized with $2 \times 10^4 p^{103-141} + \text{OVAp}$ -pulsed DC s.c./i.p., and then treated with $100 \mu\text{g}$ of antibody FGK-45. Furthermore, one group of mice received $30 \mu\text{g } p^{103-141}$, $30 \mu\text{g OVAp}$ in liposomes and pulsed onto DC, and treated with $100 \mu\text{g}$ of antibody FGK-45 as above. Two weeks later, mice were challenged in vivo according to regimens specified above. Spleen cells were collected 2–4 weeks after the second immunization and then re-stimulated in vitro uniformly with $p^{103-141}$ or OVAp in saline, respectively. Seven days later, effector cells were mixed at different E/T ratios for 4 h with ^{51}Cr -labeled IgE-secreting B4 hybridoma cells (H-2^b) (A); $p^{103-141}$ -pulsed EL-4 (H-2^b, incubated overnight with IFN- γ) (B); IgE-secreting B4 hybridomas (H-2^b) (C); and 26.82 line 14 IgE-secreting hybridomas (H-2^d) (D).

lysis at E/T 100:1), suggesting IgE peptides naturally processed and presented by B4 may be present in the same density as that pulsed on the targets [36] (Fig. 3B). Second, self-tolerance to IgE does not play a role in regulating the magnitude of CTL responses in so far as the costimulation may be optimally provided to breaking self-IgE tolerance. Thus Fig. 3C showed that similar levels of CTL-induced lysis were observed in IgE $+/+$ and in IgE $-/-$ mice (33–48% at E/T 100:1, vs. Fig. 3A). Third, IgE peptide-specific CTL exhibit MHC I-restricted cytotoxicity of target cells expressing natural IgE peptides. Thus, CTL raised in 129 IgE $+/+$ mice lysed IgE-producing B4 (H-2^b) hybridomas (Fig. 3C), but did not lyse IgE-producing 26.82 hybridomas (H-2^d) (Fig. 3D). Reciprocally, CTL obtained from IgE peptide-immunized BALB/c mice lysed IgE-secreting 26.82, but not IgE-secreting B4 hybridomas (not shown).

3.4. CTL induced by peptides inserted into CDR3 of immunoglobulin vector

We wish to determine whether IgE peptides may be protected and rendered a stable antigen in the context of a recombinant protein. We therefore compare the immuno-

genicity of a 17-mer synthetic $p^{107-123}$ comprising core overlapping $K^b/K^d/D^b$ motifs with two additional amino acids on each side, with the corresponding oligonucleotide of $p^{107-123}$ cloned into the CDR3 loop of an immunoglobulin variable domain [23,24]. As shown before, peptides expressed in antigenized antibodies (Ab^{Ag}) are immunogenic due to the longer half-life of immunoglobulin and their ability to target professional APC via the rodent IgG Fc receptor. 129/SvEv IgE $+/+$ mice (H-2^b) were immunized with $30 \mu\text{g } p^{107-123} + \text{OVAp}$ versus $30 \mu\text{g } \text{Ab}^{p^{107-123}}$. Figs. 4A and B showed that comparable CTL responses were developed in 129/SvEv mice immunized with peptide-antigenized antibody as well as those immunized with $p^{107-123}$ or with $p^{109-117}$. Three points are, (i) $\text{Ab}^{p^{107-123}}$ flanked by native CDR3 sequences may be efficiently processed and presented via the exogenous MHC I pathway [23] by APC in eliciting IgE peptide-specific CTL. (ii) Comparable CTL responses in $\text{Ab}^{p^{107-123}}$ versus $p^{107-123}$ suggest that formulation of $p^{107-123}$ in liposomes lends an extra stability of peptide antigens comparable to that of Ab^{Ag} . (iii) $p^{109-117}$ may be naturally processed and presented by both APC and IgE-producing plasma cells since comparable extent of CTL was observed with IgE-producing B4 hybridomas as targets versus $p^{109-117}$ -pulsed EL-4.

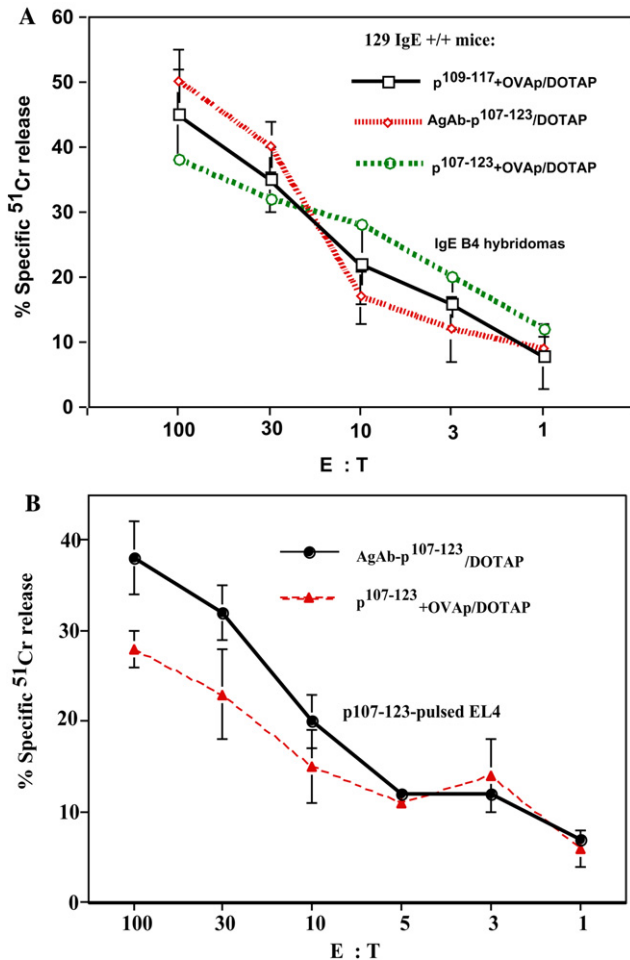


Fig. 4. Induction of IgE-specific CTL in mice with p¹⁰⁷⁻¹²³ antigenized antibody. A pair of oligonucleotides corresponding to 17-mer p¹⁰⁷⁻¹²³, encompassing K^b-binder p¹⁰⁹⁻¹¹⁷, positive for RMA-S translocation assay, was cloned into the Asp718 site of CDR3 of murine VH62 heavy chain, fused with human Fcγ [23,24]. 129/SvEv (H-2^b) wild-type mice in groups of five were immunized with 30 μg recombinant Ab^{p107-123} in DOTAP or 30 μg p¹⁰⁷⁻¹²³ or p¹⁰⁹⁻¹¹⁷ + OVAp each in DOTAP. Two weeks later, mice were boosted and spleen cells were prepared 10 days later, re-stimulated with Ab^{p107-123} and p¹⁰⁷⁻¹²³ + OVAp, respectively, in vitro for 7 days. CTL were measured against p¹⁰⁷⁻¹²³-pulsed EL-4 or IgE-secreting B4 hybridomas as targets.

3.5. CTL to natural IgE peptides suppress IgE production in vitro and in vivo

To determine whether CTL elicited by IgE peptide immunization inhibit IgE production, in vitro re-stimulated CTL were added to IgE-secreting hybridomas or LPS and IL-4 stimulated B cells in vitro. Fig. 5A showed that in vitro p¹⁰⁹⁻¹¹⁷ re-stimulated CTL from BALB/c mice immunized with p¹⁰⁹⁻¹¹⁷ inhibited 79% IgE production by 26.82 IgE-secreting hybridomas (H-2^d), while did not affect IgE production by IgE-secreting B4 hybridomas (H-2^b). Fig. 5B showed these CTL also markedly inhibited polyclonal IgE production elicited by LPS/IL-4

in vitro, e.g., 71 and 83% inhibition at E/T ratios of 10:1 and 30:1, respectively, whereas cells from control cultures did not affect IgE production. To determine the effect of IgE peptide immunization on production of allergen-specific IgE in vivo, mice were immunized with p¹⁰³⁻¹⁴¹ or p¹⁰⁹⁻¹¹⁷ with OVA peptides in liposomes twice at intervals of 2 weeks. Mice were then immunized with KLH in alum. As shown in Fig. 5C, allergen-specific IgE production was significantly ($p < 0.01$) inhibited in mice vaccinated with p¹⁰³⁻¹⁴¹ and p¹⁰⁹⁻¹¹⁷ (PCA = 20), while mice previously immunized with OVAp in liposomes or saline exhibited high titer anti-KLH IgE (PCA = 160–320). In contrast, IgG responses in IgE peptide-immunized animals remained comparable to controls (legend to Fig. 5). The inhibition of IgE production is IgE isotype-specific since KLH-specific IgG production was not affected.

4. Discussion

Previously, we described a murine model of pan-IgE deficiency induced by perinatal IgE immunization prior to the onset of self-tolerance to IgE [16–20]. Cell-mediated immunity against self-IgE appears to play a crucial role in contributing to a profound state of IgE deficiency, while circulating anti-IgE also were present to neutralize circulating IgE [16–20]. Similar IgE immunization in adult mice with preexisting circulating high levels of serum IgE, however, triggered anaphylactic responses [16,20]. We therefore hypothesize that CTL targeting IgE peptide(s) decorated MHC class I of IgE-producing cells, avoiding the sequelae of IgE–anti-IgE-mediated anaphylaxis in immunocompetent adults, may be employed as an alternative strategy to humoral anti-IgE immunity. The straightforward concept to eliminate IgE-producing cells bypasses cytokine feedback loops for Th2 development. As a result it will also obviate the risk associated with Th2 “desensitization” with allergenic CD4 T-cell epitopes, and this measure often paradoxically leads to enhanced IgE production [14,15].

It is thus pertinent to identify immunogenic IgE constant region peptides that elicit CTL eliminating/inhibiting IgE-producing cells that present naturally processed IgE peptides on MHC I of IgE-producing cells. One approach is to purify and identify natural IgE peptides directly from IgE-producing cells. This approach has yielded definitive information concerning natural peptides of unique protein antigens from tumor cell lines [37,38]. Nevertheless, this structural approach is technical demanding for equipped MS/MS laboratory, and may be limited to provide only those peptide sequences of degraded proteins present in large quantity.

A reverse approach has been undertaken recently by Zanetti [39], Melief and colleagues [40] to identify natural CTL peptides of tumor targets in a two-step

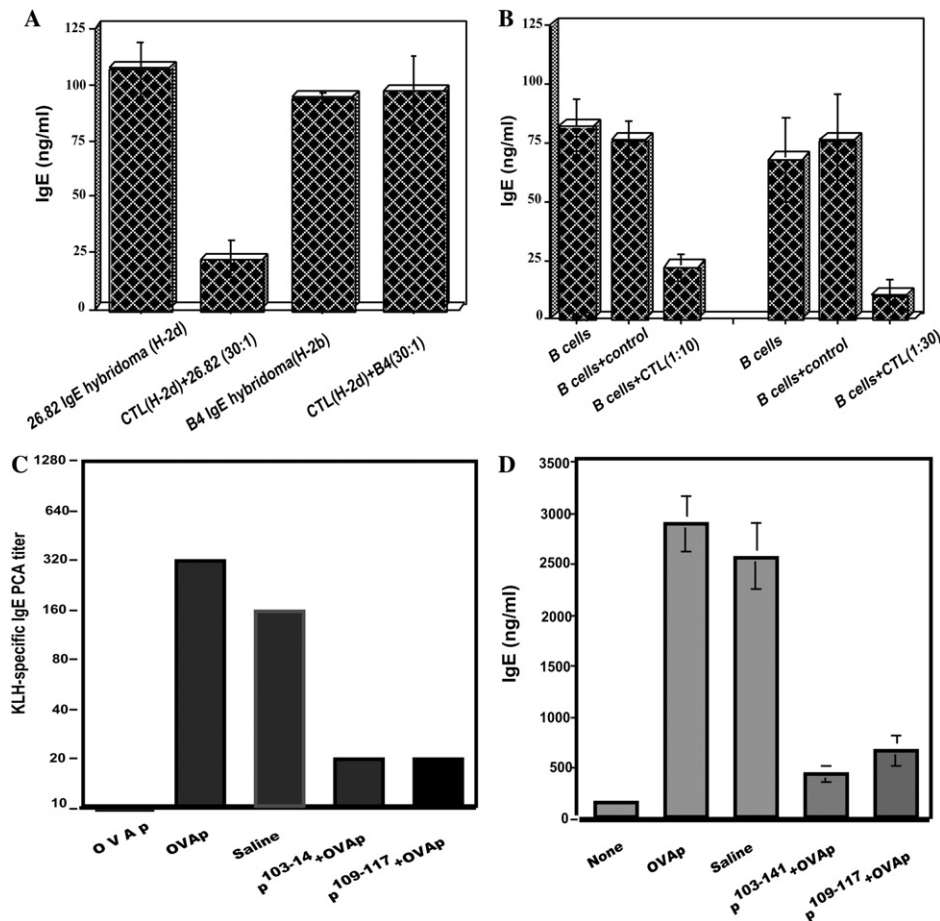


Fig. 5. In vitro and in vivo inhibition of IgE production by IgE-specific CTL. BALB/c mice (five per group) were immunized twice with p¹⁰³⁻¹⁴¹ + OVAp in DOTAP and their spleen cells re-stimulated in vitro as described above. Activated CTL were mixed with 1×10^4 IgE-secreting hybridomas, line 40 of 26.82(H-2^d) or B4 (H-2^b) at an E/T ratio of 30 to 1 (A). BALB/c splenic B cells were enriched by magnetic beads positively selected for B220. For polyclonal IgE production, 3×10^5 normal B cells were added with 3×10^6 p¹⁰³⁻¹⁴¹-activated spleen cells from mice immunized with p¹⁰³⁻¹⁴¹ + OVAp/DOTAP versus OVAp/DOTAP as a control. The co-cultures were then stimulated with 20 μ g/ml LPS and 250 U/ml murine recombinant IL-4. Two days later, the culture supernatants were harvested. IgE levels were determined using a double-sandwiched ELISA (PharMingen) (B). BALB/c mice (five per group) were immunized twice with either p¹⁰³⁻¹⁴¹, or nonameric p¹⁰⁹⁻¹¹⁷ + OVAp in liposomes, or helper peptide in liposomes at the interval of 2 weeks. Mice given saline served as control. Four days after boosting, mice were challenged with 1 μ g KLH in 2 mg alum i.p. four times at a 10-day interval, and the anti-KLH IgE (C) was then determined by passive cutaneous anaphylaxis (PCA) reaction (D). Anti-KLH IgG levels determined by antigen-specific IgG ELISA were as follows: 1540 ± 238 μ g/ml (saline); 1924 ± 520 μ g/ml (OVAp, DOTAP, $p > 0.05$); 2277 ± 638 μ g/ml (p¹⁰³⁻¹⁴¹+OVAp in DOTAP, $p > 0.05$); 1829 ± 603 μ g/ml (p¹⁰⁹⁻¹¹⁷ + OVAp in DOTAP, $p > 0.05$).

procedure. Step I: synthetic peptides corresponding to the MHC class I-binding motifs were (a) synthesized, (b) characterized by binding to MHC I molecules, and (c) antigenicity evaluated by eliciting CTL that lysed peptide-pulsed conventional targets. Step II: the protective immunogenicity was confirmed by whether the respective peptide-specific CTL were also capable of lysing physiological targets, i.e., tumor cells (or marker of a particularly differentiated cell type, such as IgE-producing cells) as targets that also endogenously express such immunogenic peptides/MHC class I complexes on cell surface [39,40]. This method of ascertainment permits delineation of protective tumor immunogenic peptide by functional immunity against tumor cells in vitro and in vivo [39,40]. Thus synthetic cytotoxic peptides, shown to be antigenic and immunogenic at once, are presumed

to correspond to physiologically processed, natural CTL peptides presented on cell surface MHC class I molecules without amino acid sequencing by MS/MS.

Since immunoglobulin peptides on immunoglobulin-secreting B and plasma cells are frequently employed as model antigens for CTL [36,39], we therefore hypothesize that p¹⁰⁹⁻¹¹⁷ exhibiting nonameric peptide motif for H-2K^d and H-2K^b may be such a natural candidate sequence. This hypothesis was tested in that (i) p¹⁰⁹⁻¹¹⁷ may be employed to elicit CTL, which were shown capable of lysing IgE-secreting hybridomas exhibiting H-2^d as well as H-2^b haplotype; and (ii) p¹⁰⁹⁻¹¹⁷ may exhibit binding to MHC I molecules. As shown in Fig. 2C, CTL from BALB/c mice (H-2^d) immunized with p¹⁰⁹⁻¹¹⁷-pulsed DC lysed not only p¹⁰⁹⁻¹¹⁷-pulsed P815 but also IgE-secreting hybridomas, 2682 of the H-2^d haplotype.

Furthermore, Fig. 2D showed that CTL from 129 mice (H-2^b) immunized with p^{109–117}, lysed p^{109–117}-pulsed EL-4 and IgE-secreting hybridoma, B4 of the H-2^b haplotype with similar efficacies. In summary, p^{109–117} indeed elicited functional CTL immunity against IgE-producing plasma cells, presumably expressing MHC class I decorated with such an endogenous IgE peptide. This observation meets the two requirements of p^{109–117} as a likely candidate for a natural IgE peptide allergy vaccine. Thus, we establish herein the parity of correspondence principle (PCP) of antigenicity (induction of IgE-specific CTL) to immunogenicity (protective recognition of IgE peptide/MHC class I on IgE-producing cells) in the current pan-IgE peptide-based vaccine model.

Observations of CTL-mediated inhibition of IgE production at the levels of IgE-producing cells both in vitro and in vivo strongly suggest that the antigenic peptide(s) within the p^{103–141}, processed and presented on MHC I rendered IgE-producing targets susceptible to CTL-mediated lysis. This demonstrated that p^{103–141}, a longer precursor peptide [16] generated by V8 (endoproteinase-Lys), indeed contained the natural cytotoxic peptide(s) participating in the afferent limb, e.g., APC for the induction of CTL and efferent limb, e.g., lysis of IgE-producing cells by such CTL. Bacterial V8 protease, employed traditionally in peptide mapping [16], may not be relevant in actual peptide processing in the rodent species. The 39-mer, p^{103–141} must therefore be captured by APC, processed by proteasomes and aminopeptidases to yield the pertinent nonameric CTL peptides for subsequent CTL induction.

DC are known to have active pinocytocytotic pathway [41,42]. Soluble IgE peptides and OVAp entrapped in liposomes are likely to enter into the phagosomal compartment, released into the cytosol. Peptide processed by cytosolic proteasomes are transported into ER and trimmed by resident aminopeptidase; consequently, these fully processed and edited peptides from p^{103–141} interact with de novo MHC I and expressed on cell surface of APC via the exocytic pathway. Nonameric p^{109–117}, on the other hand, can either bind directly on surface MHC class I. In contrast, pinocytosed OVAp were processed in endocytic vesicles, destined to intersect with the MHC containing vesicles, expressed on cell surface and OVAp and induce CD4 (+) helper T cells [39–42]. Therefore in our protocol, a single APC is presented with two separate peptide entities via two different vesicular transport pathways as well as a cell surface MHC I shunt by the same cell. This model predicts that covalent linked peptides may not be more advantageous since it requires to be later disintegrated into the discrete CTL peptide or OVAp component for different routes of vesicular trafficking into the MHC I versus the MHC II pathway, respectively.

Anti-CD40 is known to provide costimulation for CTL induction [34]. To our surprise anti-CD40

employed in this model downregulated CTL responses to the baseline (Figs. 2 and 3). This outcome may due to different routes of anti-CD40 injection. In contrast to intravenous injection by Schoenberger and colleagues [34], we administered similar doses of antibodies intraperitoneally. It is possible that activating CD40 (+) peritoneal macrophages inhibit CTL induction by peptide/liposome-pulsed DC or peptide/liposomes complexes alone [41,42]. Activated macrophages produced redox species of tryptophan metabolites that inhibited induction of CTL and thus prevented rejection of fetus [43,44]. Recently, suppression of DC was found mediated by macrophages [41,42]. In this vein, downregulation of IgE peptide-specific CTL following alleviation of allergic syndromes may actually benefit the patients for general immune protection function of IgE. In particular, IgE is known in parasitic defense and recently a beneficial role is also proposed for protecting against cancers in the rodent models [45,46].

Passive administration of anti-IgE monoclonal antibodies (Xolair) was recently introduced in the market to treat severe cases of allergic asthma. Free circulating IgE can be reduced 95% in patients undergoing 300–500 mg treatment regimens [46]. Based on the primary endpoint of asthma exacerbation, 10–12% patients (after subtracting high percentages of benefit due to the placebo effect) were improved from the anti-IgE (Omalizumab) treatment [46]. According to the secondary end point of steroid reduction, more patients (17–21%) become less dependent on inhaled steroid (but not on oral and injected steroid) during the steroid reduction phase. Notably, patients benefited from the anti-IgE antibody treatment did not show improved spirometry measurements, including FEV₁.

During the treatment, neutralized IgE persist in circulation as small sizes of anti-IgE/IgE immune complexes with increased natural half-life of 2 days pre-treatment versus 14 days post-treatment. Consequently, cumulative levels of IgE (in the form of immune complexes) in treated patients were elevated 10-fold higher than that prior to the treatment [47,48]. The concept of CTL immunization resides at the central level of IgE production owing to eliminating or inhibiting IgE-producing cells. CTL-based immunotherapy has been conducted in myeloma patients immunized with tumor idiotype-pulsed DC [49]. Analogously, targeting IgE-producing cells by CTL may be similar to targeting tumor cells bearing a unique differentiation antigen, including immunoglobulin idiotype(s) of myelomas [49–52]. To achieve greater efficacies, CTL-based therapy that inhibits central IgE production may then be combined with anti-IgE antibody-based therapy that neutralizes peripheral circulating IgE.

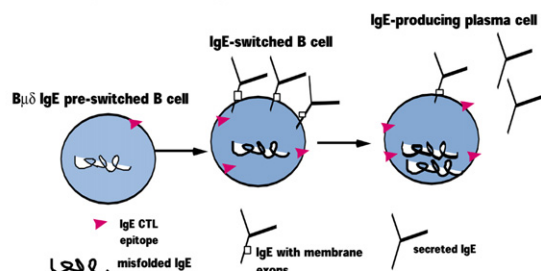
The results herein support the hypothesis of a universal pan-IgE vaccine made from constant CH₂ domain in categorically treating IgE-mediated allergic diseases regardless of their respective allergen specificities.

The observation of downregulated KLH-specific IgE responses as well as downregulated polyclonal IgE responses also in vivo in mice immunized with the “universal” allergy peptide vaccine derived from murine CH ϵ 2 substantiated this hypothesis (Fig. 5). Herein a theory of homeostatic control of steady state levels of IgE was depicted in Fig. 6. This theory proposes that IgE-producing cells may be targets of immunosurveillance by IgE peptide-specific CTL, which escape self-tolerance and regulate normal levels of central IgE production. Fig. 6A shows that such IgE-producing B cells and their precursors are at various stages of differentiation. Pre-switched B cells produce IgE without necessarily undergoing deletional gene arrangement [53]. Switched B cells with rearranged epsilon gene express surface IgE with membrane exons [54,55], while IgE-

secreting plasma cells secrete IgE of different isoforms [56]. Five or more alternatively spliced mRNA, encoding translated IgE products of different lengths, were found in U266 and SKO7 IgE plasma cells as well as allergen-stimulated human PBL [58]. Within these B cells, properly folded IgE molecules may be secreted or assembled on cell surface while denatured and/or misfolded IgE heavy chains as well as aberrant IgE heavy chain isoforms, resulting from the alternatively spliced, incomplete messages, are degraded, processed, and presented as surface antigenic peptides/MHC I complexes [23,28,39,40,57]. Fig. 6B showed that naturally degraded IgE peptides are decorated onto MHC I of IgE B cells and plasma cells. And breaking tolerance to self-antigens with a strong second helper signal may be employed as a therapeutic strategy to elicit natural IgE peptide-specific CTL that eliminate or inactivate IgE-committed B/plasma cells, consequently de novo IgE synthesis via their IgE self-markers as a natural target.

In conclusion, the data presented herein showed that active immunization with rodent IgE MHC I-restricted IgE peptide(s) leads to a profound downregulation of IgE response in vivo. This study suggests targeting a natural human self-IgE peptide/MHC I complex on human IgE-producing B and plasma cells may also inhibit human IgE production and ameliorate IgE-mediated allergy such as allergic asthma.

A IgE natural peptides as self markers on IgE producing cells



B Self-markers as CTL target

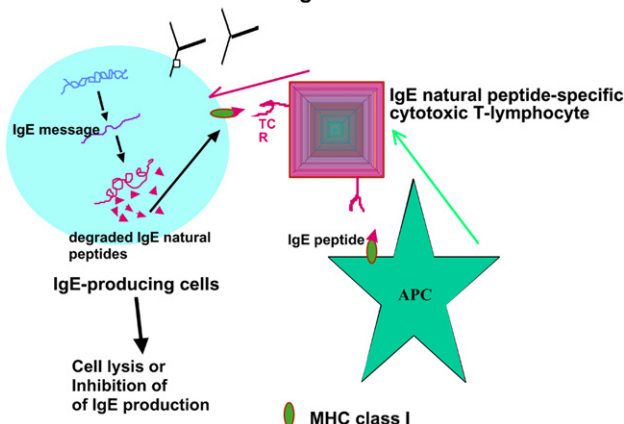


Fig. 6. Natural IgE peptides presented on MHC I of IgE B cells at different stages of differentiation are direct CTL targets. (A) Generation of natural IgE peptide fragments in IgE B cells. Pre-switch B cells produce IgE without necessarily rearranging epsilon gene; IgE-switched B cells expressed IgE heavy chain product with rearranged gene with two membrane exons (34–36). Plasma cells secrete different isoforms of IgE without membrane exons (37). Misfolded and aberrant forms of IgE are degraded and natural IgE peptides become targets of immunosurveillance. The right side of (B) depicts the vaccine concept in immunizing the host with IgE synthetic peptides corresponding to natural IgE peptides presented by MHC I molecules of APC. Cytotoxic T lymphocytes produced against a IgE-producing cell of a particular stage of differentiation halt the differentiation, inhibit IgE production, and/or lyse this IgE-producing cell.

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References

- [1] Z. Ovary, B. Benacerraf, K.J. Bloch, Properties of guinea pig 7S antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis, *J. Exp. Med.* 117 (1963) 951–964.
- [2] K. Ishizaka, Regulation of immunoglobulin E biosynthesis, *Adv. Immunol.* 47 (1989) 1–44.
- [3] M. Plaut, J.H. Pierce, C.J. Watson, J. Hanley-Hyde, R.P. Nordon, W.E. Paul, Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores, *Nature* 339 (1989) 64–67.
- [4] H. Metzger, G. Alcaraz, R. Hohman, J.P. Kinet, V. Pribluda, R. Quarto, The receptor with high affinity for immunoglobulin E, *Annu. Rev. Immunol.* 4 (1986) 419–427.
- [5] S.-S. Chen, What determines an antigen-specific IgE isotypic response?—a hypothesis, *Scand. J. Immunol.* 34 (1991) 510–519.
- [6] R.L. Coffman, J.A. Carty, T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma, *J. Immunol.* 136 (1986) 949–954.

- [7] S.-S. Chen, Q. Li, E. Pearlman, W.H. Chen, Dual mechanisms of potentiation of murine antigen-specific IgE production by cyclosporin A in vitro, *J. Immunol.* 149 (1992) 762–767.
- [8] S. Jung, K. Rajewsky, A. Radbruch, Shutdown of class switch recombination by deletion of a switch region control element, *Science* 259 (1993) 980–998.
- [9] L.A. Iciek, S.A. Delphin, J. Stavnezer, CD40 cross-linking induces Ig epsilon germline transcripts in B cells via activation of NF-kappaB: synergy with IL-4 induction, *J. Immunol.* 158 (1977) 4769–4779.
- [10] J.-F. Gauchat, D.A. Leberman, R.L. Coffman, H. Gascan, J.E. De Vries, Structure and expression of germline transcripts in human B cells induced by interleukin 4 to switch to IgE production, *J. Exp. Med.* 172 (1990) 463–473.
- [11] R.L. Coffman, D.A. Leberman, P. Rothman, Mechanism and regulation of immunoglobulin isotype switching, *Adv. Immunol.* 54 (1993) 229–270.
- [12] T.R. Mosmann, H. Cherwinski, M.W. Bond, M.A. Giedlin, R.L. Coffman, Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins, *J. Immunol.* 136 (1986) 2348–2357.
- [13] R.A. Seder, W.E. Paul, Acquisition of lymphokine-producing phenotype by CD4⁺ T cells, *Annu. Rev. Immunol.* 12 (1994) 635–673.
- [14] E.M. Janssen, M.H.M. Wauben, E.H. Jonker, G. Hofman, W. Van Eden, F.P. Nijkamp, A.J.M. Van Oosterhout, Opposite effects of immunotherapy with ovalbumin and the immunodominant T-cell epitope on airway eosinophilia and hyperresponsiveness in a murine model of allergic asthma, *Am. J. Respir. Cell Mol. Biol.* 21 (1999) 21–29.
- [15] L.J. Rosenwasser, E.W. Gelfand, Immunotherapy with antigens and epitopes: pro or con, *Am. J. Respir. Cell Mol. Biol.* 21 (1999) 4–6.
- [16] Y. Wang, R. Schmaltz, F.-T. Liu, M. Robertson, T. Petro, S.-S. Chen, Peptides derived from IgE heavy chain constant region induce profound IgE isotype-specific tolerance, *Eur. J. Immunol.* 26 (1996) 1043–1049.
- [17] S.-S. Chen, D.H. Katz, IgE class-restricted tolerance induced by neonatal administration of soluble or cell-bound IgE, *J. Exp. Med.* 57 (1983) 772–788.
- [18] S.-S. Chen, F.-T. Liu, D.H. Katz, Cellular and molecular mechanisms of murine IgE class-restricted tolerance induced by neonatal administration of soluble or cell-bound IgE, *J. Exp. Med.* 160 (1984) 953–970.
- [19] S.-S. Chen, Mechanisms of IgE tolerance: dual regulatory T cell lesions in perinatal IgE tolerance, *Eur. J. Immunol.* 21 (1991) 2461–2467.
- [20] S.-S. Chen, Genesis of host IgE competence: perinatal IgE tolerance induced by IgE processed and presented by IgE Fc receptor (CD23)-bearing B cells, *Eur. J. Immunol.* 22 (1992) 343–348.
- [21] F.-T. Liu, K. Albrandt, J.G. Sutcliffe, D.H. Katz, Cloning and nucleotide sequence of mouse immunoglobulin e chain cDNA, *Proc. Natl. Acad. Sci. USA* 79 (1982) 7850–7852.
- [22] R. Shimokevitz, S. Colon, J.W. Kappler, P. Marrack, H.M. Grey, Antigen recognition by H-2 restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen, *J. Immunol.* 133 (1984) 2067–2074.
- [23] R. Billelta, G. Filaci, M. Zanetti, Major histocompatibility complex class I-restricted presentation of influenza virus nucleoprotein peptide by B lymphoma cells harboring an antibody gene antigenized with the virus peptide, *Eur. J. Immunol.* 25 (1995) 776–783.
- [24] S. Xiong, M. Gerloni, M. Zanetti, In vivo role of B lymphocytes in somatic transgene immunization, *Proc. Natl. Acad. Sci. USA* 94 (1997) 6352–6357.
- [25] K. Inaba, J.P. Metlay, M.T. Crowley, R.M. Steinman, Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ, *J. Exp. Med.* 172 (1990) 631–640.
- [26] H.C. Oettgen, T.R. Martin, A. Wynshaw-Boris, C. Deng, J.M. Drazen, Active anaphylaxis in IgE-deficient mice, *Nature* 370 (1994) 360–367.
- [27] S.-S. Chen, Enumeration of antigen-specific IgE responses at the single-cell level by an ELISA plaque assay, *J. Immunol. Methods* 135 (1990) 129–138.
- [28] A. Townsend, C. Ohlen, J. Bastin, H.-G. Ljunggren, L. Foster, K. Karre, Association of class I major histocompatibility heavy and light chains induced by viral peptides, *Nature* 340 (1989) 443–448.
- [29] F.-T. Liu, J.W. Bohn, E.L. Ferry, H. Yamamoto, C.A. Molinaro, L.A. Sherman, N.R. Klinman, D.H. Katz, Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization, *J. Immunol.* 124 (1980) 2728–2737.
- [30] A.K. Rudolph, P.D. Burrows, M.R. Wabl, Thirteen hybridomas secreting hapten-specific immunoglobulin E from mice with Iga or Igb heavy chain haplotype, *Eur. J. Immunol.* 11 (1981) 527–529.
- [31] A. Mondin, A. Khoruts, M.K. Jenkins, The anatomy of T-cell activation and tolerance, *Proc. Natl. Acad. Sci. USA* 93 (1996) 2245–2252.
- [32] P. Bretscher, M.A. Cohn, A theory of self-nonsel discrimination, *Science* 169 (1970) 11042–11049.
- [33] J. Banchereau, R.M. Steinman, Dendritic cells and the control of immunity, *Nature* 392 (1998) 245–252.
- [34] S.P. Schoenberger, R.E. Toes, E.L. van der Voort, R. Offringa, C.J. Melief, T-cell help for cytotoxic T lymphocytes is mediated by CD40–CD40L interactions, *Nature* 393 (1998) 480–483.
- [35] J.P. Ridge, F. Di Rosa, P.A. Matzinger, A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell, *Nature* 393 (1998) 474–478.
- [36] A. Franco, D.A. Tilly, L. Gramaglia, M. Croft, L. Cipolla, M. Meldal, H.M. Grey, Epitope affinity for MHC class I determines helper requirement for CTL priming, *Nat. Immunol.* 1 (2000) 145–150.
- [37] R.M. Chic, R.G. Urban, J.C. Gorga, D.A. Vignali, W.S. Lane, J.L. Strominger, Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles, *J. Exp. Med.* 178 (1993) 27–47.
- [38] O. Rotzschke, K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, H.G. Rammensee, Isolation and analysis of naturally processed viral peptides are recognized by cytotoxic T cells, *Nature* 348 (1990) 252–254.
- [39] B. Minev, J. Hipp, H. Firat, J.D. Schmidt, P. Langlade-Demoyen, M. Zanetti, Cytotoxic T cell immunity against telomerase reverse transcriptase in humans, *Proc. Natl. Acad. Sci. USA* 97 (2000) 4796–4801.
- [40] J.H. Kessler, N.J. Beekman, S.A. Bres-Vloemans, P. Verdijk, P.A. Van-Veelen, A.M. Kloosterman-Joosten, D.C. Vissers, G.J. Ten-Bosch, M.G. Kester, A. Sijts, J. Wouter-Drijfhout, F. Ossendorp, R. Offringa, C.J. Melief, Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis, *J. Exp. Med.* 193 (2001) 73–88.
- [41] G. Rotta, E.W. Edwards, S. Sangaletti, C. Benetti, S. Ronzoni, M.P. Colombo, R.M. Steinman, G.J. Randolph, M. Rescigno, Lipopolysaccharide or whole bacteria block the conversion of inflammatory monocytes into dendritic cells in vivo, *J. Exp. Med.* 198 (2003) 1253–1263.
- [42] W. Zou, J. Borvak, S. Wei, T. Isaeva, D.T. Curiel, T.J. Curiel, Reciprocal regulation of plasmacytoid dendritic cells and monocytes during viral infection, *Eur. J. Immunol.* 31 (2001) 3833–3839.
- [43] D.H. Munn, E. Shafizadeh, J.T. Attwood, I. Bondarev, A. Pashine, A.L. Mellor, Inhibition of T cell proliferation by macrophage tryptophan catabolism, *J. Exp. Med.* 189 (1999) 1363–1372.
- [44] D.H. Munn, M. Zhou, J.T. Attwood, I. Bondarev, S.J. Conway, B. Marshall, C. Brown, A.L. Mellor, Prevention of allogeneic fetal rejection by tryptophan catabolism, *Science* 281 (1998) 1191–1193.
- [45] S.N. Karagiannis, Q. Wang, N. East, F. Burke, S. Riffard, M.G. Bracher, R.G. Thompson, S.R. Durham, L.B. Schwartz, F.R. Balk-

- will, H.J. Gould, Activity of human monocytes in IgE antibody-dependent surveillance and killing of ovarian tumor cells, *Eur. J. Immunol.* 33 (2003) 1030–1040.
- [46] Advisory Committee Clinical Efficacy Briefing Document, Genentech, Inc., Biologics Marketing Application STN 103976/0: Omalizumab for asthma, DHHS. PHS. FDA (2003) pp. 1–118.
- [47] J. Corne, R. Djukanovic, L. Thomas, J. Warner, L. Botta, B. Grandordy, D. Gygax, C. Heusser, F. Patalano, W. Richardson, E. Kilchherr, T. Staihelin, F. Davis, W. Gordon, L. Sun, R. Liou, G. Wang, T.W. Chang, S. Holgate, The effect of intravenous administration of a chimeric anti-IgE antibody on serum IgE levels in atopic subjects: efficacy, safety, and pharmacokinetics, *J. Clin. Invest.* 99 (1997) 879–887.
- [48] T.W. Chang, The pharmacological basis of anti-IgE therapy, *Nat. Biotechnol.* 18 (2000) 157–162.
- [49] M.V. Dhodapkar, J. Krasovsky, K. Olson, T cells from the tumor microenvironment of patients with progressive myeloma can generate strong, tumor-specific cytolytic responses to autologous, tumor-loaded dendritic cells, *Proc. Natl. Acad. Sci. USA* 99 (2002) 13009–13013.
- [50] P. Van der Bruggen, J. Bastin, T. Gajewski, P.G. Coulie, P. Boel, C. De Smet, C. Traversari, A. Townsend, T. Boon, A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3, *Eur. J. Immunol.* 24 (1994) 3038–3043.
- [51] I. Hara, Y. Takechi, A.N. Houghton, Implicating a role for immune recognition of self in tumor rejection: passive immunization against the brown locus protein, *J. Exp. Med.* 182 (1995) 1609–1614.
- [52] Y.L. Wen, R. Min, G. Trichot, B. Barlogie, Q. Yi, Idiotype-specific cytotoxic T lymphocytes in multiple myeloma: evidence for their capacity to lyse autologous primary tumor cells, *Blood* 99 (2002) 3280–3285.
- [53] T. MacKenzie, H.-M. Dosch, Clonal and molecular characteristics of the human IgE-committed B cell subset, *J. Exp. Med.* 169 (1989) 407–430.
- [54] L.M. Yu, C. Peng, S.M. Starnes, R.S. Liou, T.W. Chang, Two isoforms of human membrane-bound alpha Ig resulting from alternative mRNA splicing in the membrane segment, *J. Immunol.* 145 (1990) 3932–3936.
- [55] K. Zhang, A. Saxon, E.E. Max, Two unusual forms of human immunoglobulin E encoded by alternative RNA splicing of e heavy chain membrane exons, *J. Exp. Med.* 176 (1992) 233–246.
- [56] K. Zhang, E.E. Max, H.-K. Cheah, A. Saxon, Complex alternative RNA splicing of e-immunoglobulin transcripts produces mRNAs encoding four potential secreted protein isoforms, *J. Biol. Chem.* 269 (1994) 456–462.
- [57] K.L. Rock, A.L. Goldberg, Degradation of cell proteins and the generation of MHC class I-presented peptides, *Annu. Rev. Immunol.* 17 (1999) 739–779.
- [58] F.D. Batista, D.G. Efremov, O.R. Burrone, Characterization of a second secreted IgE isoform and identification of an asymmetric pathway of IgE assembly, *Proc. Natl. Acad. Sci. USA* 93 (1996) 3399–3404.