

INHIBITION OF ANTIGEN-SPECIFIC IgE PRODUCTION BY ANTIGEN COUPLED TO MEMBRANE IgE PEPTIDE ¹

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ABSTRACT

The membrane IgE peptide (MEP) encompassing 20 amino acids proximal to the C terminus of membrane IgE molecules, and secretory IgE peptides (SEP), spanning CH ϵ 1 to 4 domain were synthesized according to IgE genomic and cDNA sequences. Inhibition of anti-KLH and anti-BGG IgE, but not IgG responses was observed in mice treated with MEP-protein but not SEP-protein conjugates in complete/incomplete Freund's adjuvant. Only IgE responses directed toward proteins to which MEP was conjugated, were inhibited, while IgE responses to a concomitantly injected, unrelated antigen were not. Inhibition of antigen-specific IgE was also not correlated with levels of anti-MEP or anti-IgE antibodies; moreover, levels of total IgE remained comparable among mice treated with MEP-protein conjugates, native or glutaraldehyde-modified protein carriers. This observation may have significant import on future design of IgE immunotherapy. Treatment of MEP conjugated allergens prevents formation of IgE-anti-IgE complexes because the MEP sequence is absent from the secretory IgE.

INTRODUCTION

Although Th2 helper cells and their cytokines are known to contribute to primary and polyclonal IgE responses (1-3), studies of perinatal IgE tolerance strongly suggest the existence of another subset of helper T cells, required for antigen-specific IgE production (3-8). This subset of T cells is capable of recognizing processed IgE ligand, and its development is influenced by perinatal exposure to IgE peptides presented by CD23+ B cells (5-7, 9). Recently the crucial amino acid sequences present on CH ϵ 2 to CH ϵ 4 domains of the secretory molecule, required for regulating IgE responses, were elucidated (8). These observations prompt the

question whether amino acids encoded in membrane IgE molecule also impart a regulatory role to IgE synthesis.

Compared to secretory IgE, membrane IgE also consists of a unique stretch of 20 amino acids N-terminal to the fourth CH ϵ domain, as membrane proximal IgE connecting peptide (MEP), a short hydrophobic membrane anchor, and the cytoplasmic domain (10, 11). As predicted from the genomic IgE DNA sequence, eight out of twenty amino acid residues within the MEP are unique to the IgE isotype (10, 11). Thus we tested the hypothesis that the extracellular MEP peptide may serve as a regulatory site for IgE responses (12, 13). Herein, we reported that suppression of antigen-specific IgE responses in mice treated with MEP conjugated antigens, indicating the existence of a regulatory determinant in the MEP region of the membrane IgE molecule. Since MEP is absent from the secretory IgE molecule, MEP conjugated allergens may be safely employed to desensitize allergen-specific IgE-mediated allergic diseases.

MATERIALS AND METHODS

Reagents and chemicals: DNA sequences of IgE genomic and cDNA were employed (10, 11). Membrane proximal IgE connecting peptide (MEP), selected from the IgE genomic sequence: ELDIQDLCEIEVEGEELEEL (MEP) was synthesized at Tanox Biosystems, Inc (Houston, TX). Secretory IgE peptides (SEP) from CH ϵ 1 to CH ϵ 4 domains were prepared by Cambridge Peptide Co. (Cambridge, MA): TTSQVTSWGKSAKNFTCHVT (SEP-A: residue 190-210 of CH ϵ 1), GVDYLAHTR (SEP-B: residue 316-324 of CH ϵ 2), PLDLYQNGAC (SEP-C: residue 343-351 of CH ϵ 3). IgE peptides at 5 mg/ml were mixed with insulin B chain, BSA, KLH respectively, at 2 mg/ml in equal volumes to which glutaraldehyde was added at a final 0.05%, incubated at 25°C for 4 hr, and dialyzed. Monoclonal rat anti-mouse IgE: EM 95 and BF815 were employed for total IgE assay (14). Biotinylated rat anti-mouse kappa was obtained from Zymed (San Francisco, CA). Affinity pure biotinylated goat anti-mouse IgG, IgG1, IgG2a, IgG2b were obtained from Southern Biotechnology Associates (SBA, Birmingham, AL). P-nitrophenyl phosphate (PNPP) was obtained from Sigma Co. (St. Louis, MO). Streptavidin-alkaline phosphatase (SA-AP) was obtained from Zymed.

Source of animals: Eight week old female BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were grouped in six, and were treated with MEP-coupled to protein carriers according to the protocols described in legends to Tables and Figures. Sera

were collected on day seven after the last immunization. Antigen-specific IgE was assessed by passive cutaneous anaphylactic (PCA) skin test (4).

ELISA: Anti-MEP assay: 50 μ l MEP-BSA at 10 μ g/ml were coated onto 96-well plate at 37°C for 1 hr; the plates were washed, blocked with Blotto, and added with 50 μ l serum samples at appropriate dilutions. The plates were washed, incubated with biotinylated goat anti-mouse IgG or IgG subclasses, at 1 μ g/ml for 1 hr at r.t., washed, added with SA-AP, substrate, and read at 414 nM. Total IgE sandwich assay (14): 96-well plates were coated with 50 μ l MAb anti- ϵ , EM95, at 10 μ g/ml overnight at 4°C, washed, blocked, added with sera at appropriate dilutions, biotinylated MAb anti- ϵ , BF815 was added, and plates developed as above. Anti-IgE assay (4): anti-NP IgE (λ , ϵ) was used to coat the 96-well plates at 10 μ g/ml overnight at 4°C; the plates were washed and blocked. Sera were added at appropriate dilutions, washed, followed by biotinylated rat anti-mouse kappa light chain, and developed as above.

RESULTS

MEP conjugated protein administered in CFA/ICFA inhibited IgE responses to the carrier protein.

Adult BALB/c mice were immunized five times i.p. with MEP-KLH conjugates in complete and incomplete Freund's adjuvant (CFA/ICFA), or in alum. Anti-KLH IgE responses were assessed in individual mice. As shown in Figure 1, normal magnitude of anti-KLH IgE responses was observed in mice immunized i.p. with 10 μ g KLH in CFA/ICFA, or in alum (Gr1 & Gr4). In contrast, mice treated with 1 μ g (Gr2) or 10 μ g (Gr3) MEP-KLH in CFA/ICFA exhibited profoundly suppressed KLH specific IgE responses. The adjuvant played an important role in inhibiting IgE responses to MEP conjugated protein. Thus as shown also in Figure 1, comparable anti-KLH IgE responses were elicited in BALB/c mice immunized with 10 μ g KLH (Gr4), or with 1 μ g (Gr5) or 10 μ g (Gr6) MEP-KLH in alum.

MEP conjugated antigen did not affect antigen-specific IgG responses to the carrier. As shown in Table I, comparable anti-KLH IgG1 responses were observed in KLH or MEP-KLH immunized mice (Gr1 vs Gr2-3, $p>0.05$; Gr4 vs Gr5-6, $p>0.05$), while alum adjuvant favored antigen-specific IgG1 production over CFA/ICFA (Gr1-3 vs Gr4-6, $p<0.05$). Higher levels of anti-MEP of IgG1 subclass were observed in mice immunized with MEP-KLH in alum (Gr5-6

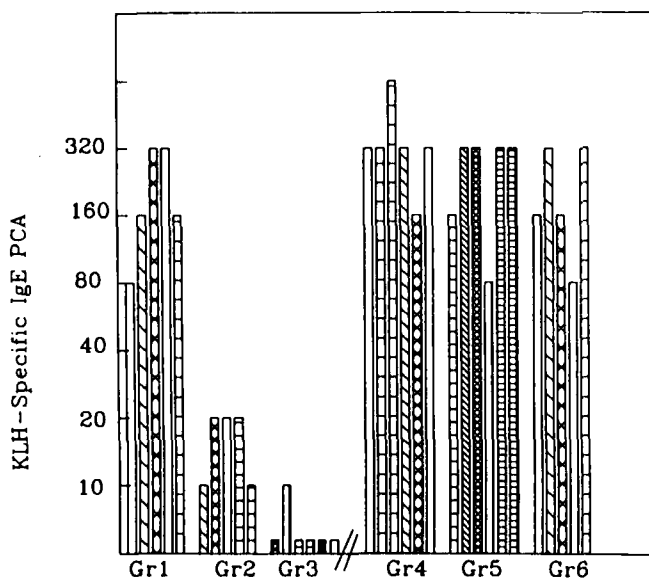


FIGURE 1

Inhibition of anti-carrier IgE responses in mice immunized with MEP conjugated antigen.

Eight week old BALB/c mice were immunized five times i.p. with 10 μ g KLH as control, or 1 μ g or 10 μ g MEP-KLH in 0.5 ml CFA/ICFA (Gr1 vs Gr2&3), or in 2 mg alum (Gr4 vs Gr5&6) at intervals of ten days. Mice were bled on day seven following the fifth antigenic challenge. KLH-specific IgE responses were assessed in individual mice by PCA.

vs Gr2-3, $p < 0.05$). In contrast, anti-KLH and anti-MEP of IgG2a and IgG2b subclasses were present in higher concentrations in mice immunized with MEP-KLH in CFA/ICFA (Gr3 vs Gr6, $p < 0.05$). However, suppression of anti-KLH IgE responses (Figure 1) appeared not directly correlated with the levels of different subclasses of anti-MEP IgG antibodies. Although anti-KLH IgE was suppressed in MEP-KLH treated mice, total IgE levels appeared to be normal in mice immunized with MEP-KLH emulsified in CFA/ICFA.

To ascertain that suppression of KLH responses was not due to alteration of protein carrier by chemical coupling, synthetic peptides corresponding to CH ϵ 1 to CH ϵ 4 domains, as well as insulin B chain (InB), were coupled to KLH by glutaraldehyde under similar conditions. As shown in Figure 2, comparable magnitude of anti-KLH IgE responses was observed in mice immunized with KLH coupled to SEP A-C or InB in CFA/ICFA, whereas anti-KLH IgE responses were suppressed in mice treated with MEP-KLH.

TABLE I: Induction of anti-carrier and anti-MEP antibodies versus total IgE in MEP conjugated antigen immunized mice.

	Treatment	Anti-KLH IgG1 ($\mu\text{g/ml}$)	Total IgE (ng/ml)	Anti-MEP IgG1 ($\mu\text{g/ml}$)	Anti-MEP IgG2a ($\mu\text{g/ml}$)	Anti-MEP IgG2b ($\mu\text{g/ml}$)
Gr1	KLH, 10 μg , CFA/ICFA	1209 $\pm$ 245	228 $\pm$ 25	<1	<1	<1
Gr2	MEP-KLH, 1 μg , CFA/ICFA	953 $\pm$ 235	138 $\pm$ 27	25 $\pm$ 10	135 $\pm$ 30	126 $\pm$ 26
Gr3	MEP-KLH, 10 μg , CFA/ICFA	1074 $\pm$ 327	148 $\pm$ 34	176 $\pm$ 45	854 $\pm$ 212	471 $\pm$ 121
Gr4	KLH, 10 μg , alum	4625 $\pm$ 1680	62 $\pm$ 20	<1	<1	<1
Gr5	MEP-KLH, 1 μg , alum	5190 $\pm$ 1529	84 $\pm$ 23	580 $\pm$ 200	121 $\pm$ 45	82 $\pm$ 16
Gr6	MEP-KLH, 10 μg , alum	8825 $\pm$ 2472	90 $\pm$ 29	375 $\pm$ 120	38 $\pm$ 38	25 $\pm$ 12

BALB/c mice were treated with MEP-KLH in CFA/ICFA, or in alum as described in Legend to Figure 1. The fifth anti-KLH IgG1 responses, total IgE levels, and anti-MEP of IgG subclasses were measured by ELISAs as described in Materials and Methods. Two-tailed, Student t-test were performed among groups with the significance level at 0.05.

Suppression of IgE responses to MEP conjugated proteins did not affect a concomitant unrelated antigenic challenge.

To examine whether MEP-KLH treatment may suppress IgE responses to an unrelated antigen, mice were pretreated with 1 to 50 μg MEP-KLH in CFA/ICFA (Gr5, 6, 8), or with 10 μg MEP-KLH in CFA twice (Gr7), followed by a challenge with MEP-KLH along with OVA in ICFA, and further boosted with OVA/MEP-KLH in ICFA twice. As shown in Figure 3, MEP-KLH administered in CFA/ICFA, inhibited IgE responses against the KLH to which MEP was coupled (3rd and 5th responses) but did not inhibit IgE responses against an unrelated OVA antigenic challenge (Gr5-8). In contrast, mice treated with glutaraldehyde-modified KLH from 1 to 50 μg in CFA/ICFA exhibited normal levels of anti-KLH, and anti-OVA IgE responses (Gr1-4).

To test whether suppression of MEP-KLH may be extended to other MEP conjugated antigens, BALB/c mice were injected with 20 μg soluble MEP-BGG or glutaraldehyde modified

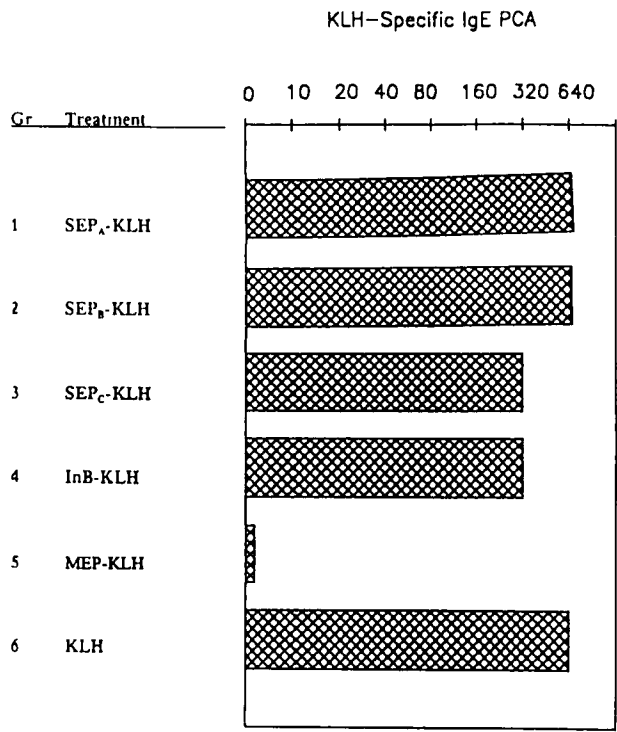


FIGURE 2

Effect of peptide conjugation on anti-carrier IgE responses. BALB/c mice were immunized i.p. five times with 10 µg SEP-KLH, InB-KLH, or MEP-KLH, or KLH as control in CFA/ICFA at intervals of ten days, sera were collected, and assessed for KLH-specific IgE responses by PCA.

BGG (GA-BGG) subcutaneously (Gr1, 3, 5, 7), or intraperitoneally (Gr2, 4, 6, 8). Mice were then challenged with MEP-BGG plus OVA (Gr1-4), or BGG plus OVA (Gr5-8) in alum. As shown in Figure 4, mice treated with soluble MEP-BGG via either route, failed to elicit anti-BGG IgE when challenged with MEP-BGG or BGG (Gr1-2 & Gr5-6) in alum, whereas anti-OVA IgE responses in these mice were normal. As a control, treatment with GA-BGG via either route did not affect subsequent anti-BGG or anti-OVA IgE responses (Gr3-4 & Gr7-8).

Moreover, as shown in Table II, MEP conjugated BGG did not affect anti-BGG IgG responses. Comparable anti-BGG or anti-OVA IgG responses were observed in mice treated with MEP-BGG and GA-BGG. Anti-MEP IgG was not detectable in mice treated with soluble MEP-BGG (Gr1-2 vs 3-4 and Gr5-6 vs 7-8, $p>0.05$). MEP-BGG treatment did not augment the

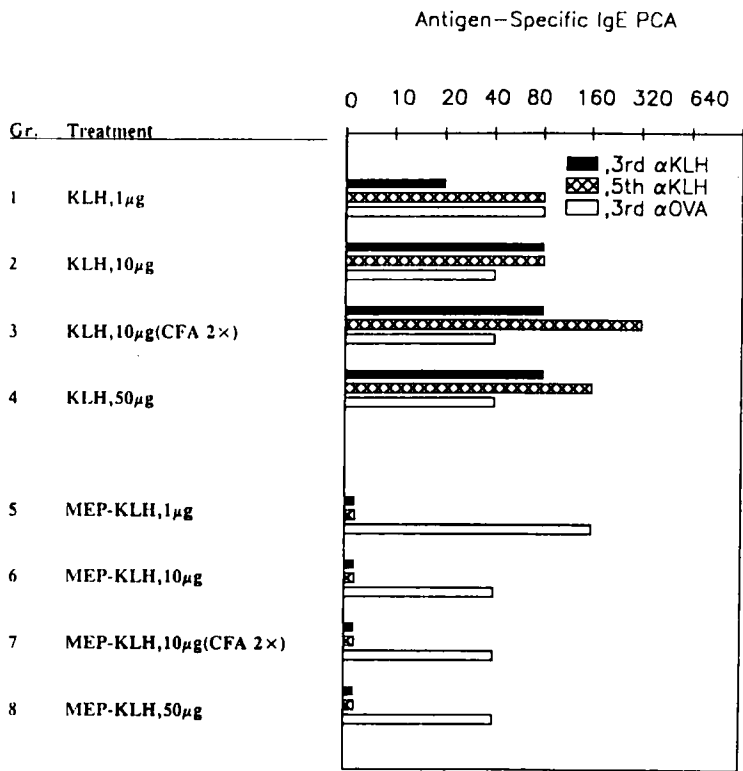


FIGURE 3

IgE responses to concomitant OVA challenge were normal in mice treated with MEP-KLH in CFA/ICFA. BALB/c mice were immunized i.p. at 10 days intervals with MEP-KLH from 1 to 50 μg in CFA followed by ICFA, or immunized with 10 μg MEP-KLH in CFA twice. Mice were then further boosted with OVA/MEP-KLH in ICFA twice. Sera were collected, and anti-KLH and anti-OVA IgE were assessed by PCA.

production of anti-IgE nor modulate the levels of total IgE levels. Moreover, as shown in Table II, total IgE as well as basal levels of anti-IgE antibodies were also comparable in mice treated with MEP-BGG or BGG as control (Gr1-2 vs 3-4 and Gr5-6 vs 7-8, $p>0.05$).

DISCUSSION

Herein we demonstrated that inhibition of antigen-specific IgE production can be achieved by treatment with MEP conjugated antigens. Three salient features are: a) Inhibition of anti-KLH and anti-BGG IgE, but not IgG responses was observed in mice treated with soluble

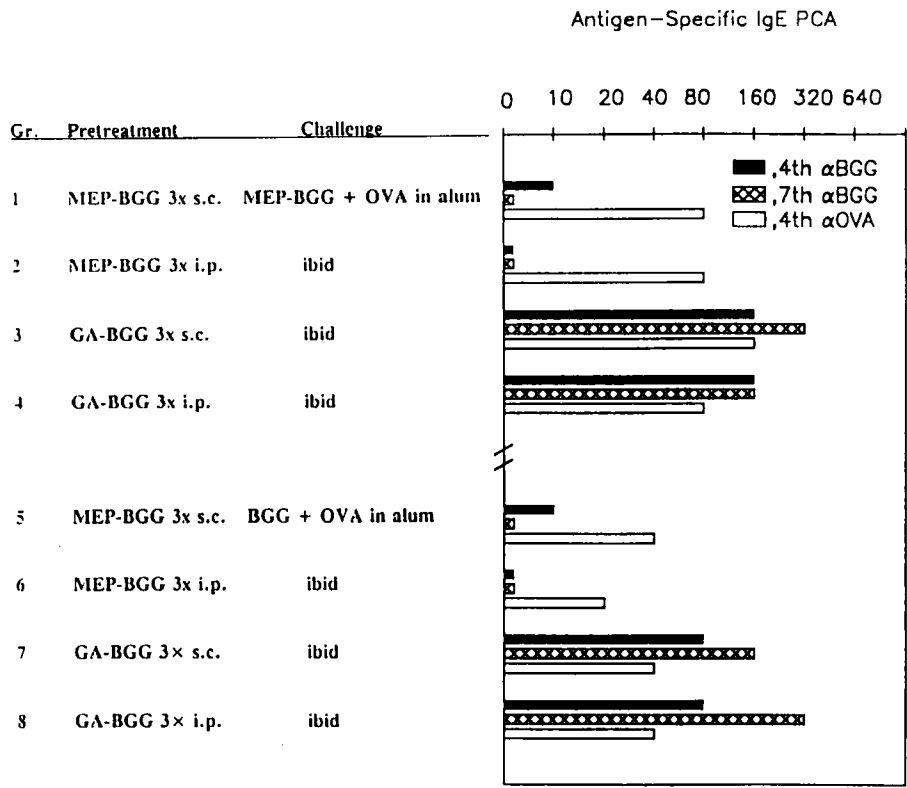


FIGURE 4

Inhibition of anti-BGG IgE responses by soluble MEP-BGG treatment. BALB/c mice were pretreated three times s.c. or i.p. with 20 µg soluble MEP-BGG or glutaraldehyde treated BGG (GA-BGG) at 10 days intervals. Mice were then challenged seven times with either 20 µg MEP-BGG/10 µg OVA or with 10 µg BGG/10 µg OVA in 2 mg alum. The fourth and seventh anti-BGG and anti-OVA IgE responses were assessed by PCA.

or MEP-conjugated protein emulsified in CFA/ICFA. b) Inhibition was observed in IgE responses to MEP conjugated carrier protein, but not toward an unrelated antigen. c) Inhibition of antigen-specific IgE was not correlated with levels of anti-MEP or anti-IgE antibodies; moreover, total IgE levels remained comparable among mice treated with MEP conjugated antigens, native or glutaraldehyde-modified carrier antigen as control.

Three interpretations are in order. First, treatment with MEP conjugated antigen may induce anti-MEP antibodies which recognize native MEP determinants on IgE committed B cells. Its inhibitory effect may be augmented when MEP conjugated antigen is also focused by IgE

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TABLE II: Antibody responses in MEP-BGG pretreated mice

	Pretreatment, 3x	Challenge in alum	IgG anti- BGG	IgG anti- OVA	IgG anti- MEP	Anti- IgE	IgE
Gr1	MEP-BGG, s.c.	MEP-BGG + OVA	915.7 ±153 (µg/ml)	72 ±21 (µg/ml)	< 1 (µg/ml)	0.173 (OD ₄₁₄)	640 ±176 (ng/ml)
Gr2	MEP-BGG, i.p.	ibid	1008.8 ±345	48 ±14	<1	0.193	706 ±213
Gr3	GA-BGG, s.c.	ibid	1470 ±423	64 ±24	<1	0.188	858 ±256
Gr4	GA-BGG, i.p.	ibid	1119 ±398	58 ±21	<1	0.145	840 ±321
Gr5	MEP-BGG, s.c.	BGG+OV A	855 ±231	82 ±40	<1	0.116	758 ±280
Gr6	MEP-BGG, i.p.	ibid	988 ±120	74 ±12	<1	0.219	706 ±246
Gr7	GA-BGG, s.c.	ibid	1215 ±258	95 ±21	<1	0.223	850 ±278
Gr8	GA-BGG, i.p.	ibid	798 ±199	80 ±13	<1	0.121	1008 ±423

BALB/c mice were treated with MEP-BGG as described in Legend to Figure 4. Levels of anti-BGG, anti-OVA upon the fourth challenge; and those of anti-MEP, anti-IgE antibodies, and total IgE upon the seventh challenge, were measured as described in Materials and Methods. Two-tailed, Student t-test were performed among groups with the significance level at 0.05

committed B cells via membrane IgE molecules. This subset of efficacious anti-MEP may escape detection by the current ELISA assay, employing MEP conjugated BSA as the coating reagent which did not exhibit native MEP conformation. Second, MEP conjugated antigens may interfere with development of IgE isotype-specific helper T cells. Previously we showed that development of a subset of CD4⁺ T cells required for antigen-specific IgE responses, was inhibited by perinatal injection of IgE peptides presented by CD23⁺ B cells (4-9). It is possible that immunization of MEP conjugated antigens may affect the presentation of regulatory IgE peptides by IgE committed B cells which in turn inhibit development of CD4⁺ helper T cells required for antigen-specific IgE production. Third, MEP conjugated antigens may preferentially induce antigen-specific Th1 cells which inhibit antigen-specific IgE responses (2, 15, 16). A polarized dichotomy of Th1/Th2 in favor of Th1, is consistent with the observations that MEP conjugated antigens emulsified in CFA/ICFA preferentially stimulated antigen-specific IgG2a and IgG2b production, while that delivered in alum preferentially stimulated IgG1 responses (Table I).

The observation reported herein may be pertinent for studying a pathophysiological role of MEP in regulating IgE production in IgE committed B cells. Our findings also have significant import for IgE immunotherapy in that allergen-desensitization may be achieved without risk of producing IgE/anti-IgE complexes which trigger mast cell degranulation, because the MEP determinant is not present on the allergen-specific secretory IgE molecule (10, 11).

ABBREVIATIONS

CFA/ICFA, complete/incomplete Freund's adjuvant; GA, glutaraldehyde; GA-KLH or GA-BGG, glutaraldehyde modified KLH or BGG; InB, insulin B chain; MAb anti- ϵ , monoclonal antibody against mouse IgE; MEP, membrane IgE peptide; PCA, passive cutaneous anaphylaxis assay; PNPP, p-nitrophenyl phosphate; SEP, soluble IgE peptide.

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