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Linkage of Ii-Key segment to gp100(46–58) epitope enhances the production of epitope-specific antibodies

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Abstract

Linkage of the Ii-Key segment of the Ii protein to MHC class II epitope gp100(46–58) using a polymethylene linker significantly enhances the production of epitope-specific antibodies in HLA-DR4-IE transgenic mice. This enhancement is not restricted by the spacer length in between the Ii-Key and epitope. The use of either IFA or CFA induced only epitope-specific IgG1. In contrast, CpG adjuvant induced both IgG1 and IgG2a isotypes. These results indicate that the Ii-Key hybrid technology is a novel and potent method to increase the immunogenicity of a MHC class II epitope. It can also be used to more efficiently generate epitope-specific antibodies.

Keywords: Ii-Key segment; gp100(46–58); MHC

1. Introduction

A novel technique to boost dramatically the vaccine potency of MHC class II-presented epitope peptides has been developed [1–3]. Binding of the Ii-Key portion of the Ii protein to the allosteric site of MHC class II molecules induces a conformational change in the peptide-binding groove for more efficient epitope charging. In model in vitro systems, the enhancement in stimulation over free epitope peptide is >250 times when the N-terminus of the epitope is covalently linked through a chemical spacer to the C-terminus of the Ii-Key peptide, forming an Ii-Key/antigenic epitope hybrid [2,3]. Further, an Ii-Key/HER-2/*neu* MHC class II hybrid induced much greater IFN-γ release from PBMC of breast cancer patients than the comparable HER-2/*neu* epitope-only peptide [4]. We have also shown that an Ii-Key/HIV gag hybrid significantly enhances the potency of the gag epitope in vivo [3]. The in vivo enhancement of CD4+ T cell activation by Ii-Key was further confirmed by immunizing HLA-DR4-IE Tg mice with a series of Ii-Key/gp100(46–58) hybrids [in preparation].

In order to determine whether the Ii-Key technology can enhance the generation of epitope-specific antibodies, we have designed and synthesized a series of Ii-Key/gp100(46–58) hybrids to immunize HLA-DR4-IE Tg mice. The measurement of antibodies in sera from immunized mice indicates that Ii-Key-linked epitopes significantly enhance the production of epitope-specific antibodies.

2. Materials and methods

2.1. Animals and immunizations

HLA-DR4-IE Tg mice [5] express HLA-DRA-IE alpha and HLA-DRB1*0401-IE beta chimeric genes. Six to eight weeks old female HLA-DR4-IE Tg mice were purchased from Taconic Laboratories and housed at the University of Massachusetts animal facility. Tg mice were immunized subcutaneously at the base of the tail with 40 or 60 nmole of Ii-Key/gp100(46–58) hybrids or epitope-only peptides, dissolved in saline and emulsified with an equal volume of either IFA, CFA (Sigma), or IFA plus 60 μg of a syn-

Abbreviations: Ii, invariant chain protein; Ii-Key, a peptide from Ii protein; Tg, transgenic

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Table 1
Design of Ii-Key/gp100 (46–58) hybrids

Sequence			Abbreviation
Ac-	RQLYPEWTEAQRL	-NH_2	A
Ac-LRMK- *ava*-	NRQLYPEWTEAQRL	-NH_2	B
Ac-LRMK- *ava*-	RQLYPEWTEAQRL	-NH_2	C
Ac-LRMK- *ava*-	QLYPEWTEAQRL	-NH_2	D
Ac-LRMK- *ava*-	LYPEWTEAQRL	-NH_2	E

Ii-Key was linked to different amino acid of the N-terminus of gp100(46–58) by one *ava* (5-aminopentanoic acid) spacer. The flexible polymethylene spacer (δ-aminovaleric acid, *-ava-*) was a non-natural amino acid incorporated during peptide synthesis. All peptides were terminally acetylated and amidated to inhibit exopeptidases.

thetic phosphorothioate-modified CpG ODN 1826, 5′-TCC ATGACGTTC CTG ACGTT nucleotide (CpG motif underlined) (Oligos Etc., Wilsonville, OR). Three weeks after immunization, sera were taken and measured for IgG1, IgG2a, and IgE antibody titers by ELISA.

2.2. Synthesis of peptides

The MHC class II-restricted human gp100(46–58) epitope was originally identified in HLA-DR4-IE Tg mice by Toloukian et al. [5]. A series of Ii-Key/gp100(46–58) hybrids were synthesized (Table 1) (Commonwealth Biotechnologies, Inc., Richmond, VA). The Ii-Key core (LRMK) was linked by different residues at the N-terminus of the gp100(46–58) to one *ava* (5-aminopentannoic acid) spacer. The peptides were dissolved in sterile water (2 nmole/μl) and stored at −20 to −80 °C before use.

2.3. ELISA

Nunc immunoplates (Fisher Scientific) were coated with the gp100(46–58) peptide (20 μg/ml) in 100 μl of coating buffer (Sigma) overnight at 4 °C and then blocked for 2 h with 20% BSA and 1% Thimerosal in 1× PBS (blocking buffer). The plates were then washed three times with PBST (1× PBS with 0.05% Tween-20). Sera from immunized animals were added at 1:3 serial dilutions and incubated for 2 h at RT. After washing five times with PBST, 2 μg/ml of either biotinylated anti-mouse IgG1, IgG2a and IgE Abs (BD Pharmingen) were added and plates were incubated for 1 h at RT. The plates were then washed five times with PBST and Avidin-HRP (Southern Biotechnologies) was added at 1:2000 dilution for 1 h incubation at RT. Avidin-HRP was removed by washing five times with PBST. TMB substrate (Sigma) was used for the detection of the colorimetric reaction.

3. Results

3.1. Epitope-specific antibody titers are enhanced by Ii-Key in a spacer length-independent manner

As illustrated in Fig. 1, the gp100(46–58)-specific IgG1 titer was enhanced five to eight times by all Ii-Key gp100

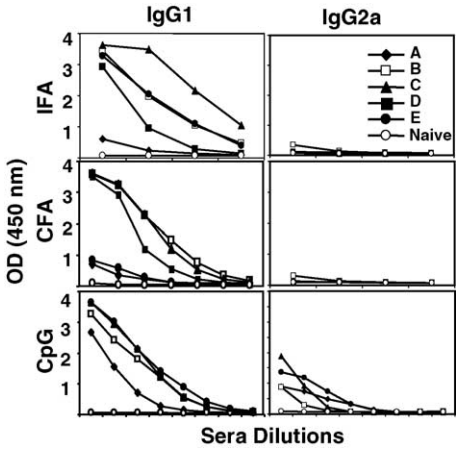

Fig. 1. Epitope-specific isotype profile induced by Ii-Key/gp100(46–58) hybrids and epitope-only vaccinations in IFA, CFA, and CpG. Vaccinations performed in IFA and CFA demonstrated stronger IgG1 response by hybrids B–D than the epitope alone. Hybrid E did enhance the IgG1 antibody production in IFA but not in CFA. IgG2a is negative in all samples. Vaccinations administered along with the CpG motif elicited a stronger IgG1 response for all hybrids. IgG2a was induced for hybrids in 4 out of 10 mice when compared to the gp100(46–58) epitope alone. Data are averaged from 5 (IFA), 25 (CFA), and 10 (CpG) mice per group. The starting dilution is 1:20 and each point is a consecutive three-fold dilution.

hybrids (except hybrid E) compared to the gp100(46–58) epitope-only peptide in both CFA and IFA. Hybrid E did not induce higher antibody titer than epitope-only in CFA. The enhancement was with a lesser variation among other hybrids, indicating a spacer length-independent enhancement fashion. This phenomenon has been observed in all samples we have tested. In the CpG group, all hybrids induced higher IgG1 titer than epitope-only which also induced good IgG1 titer.

3.2. Induction of epitope-specific IgG2a occurred only when CpG was used as adjuvant

There was no evidence of IgG2a titer when the Ii-Key/gp100 hybrids or epitope-only peptide was used in IFA or CFA. However, mice immunized with Ii-Key/gp100 hybrids plus CpG demonstrated higher gp100(46–58)-specific IgG2a titer than the gp100(46–58) epitope-only peptide plus the CpG (Fig. 1): 4 out of 10 mice in hybrid group E and 2 out of 10 mice in peptide group A induced high IgG2a titers. More boosting may be needed to stimulate all mice to have higher IgG2a titer. Epitope-specific IgE was negative in all samples from mice immunized only with IFA, CFA, and CpG.

4. Discussion

We show here that gp100(46–58)-specific antibody titers are significantly enhanced by Ii-Key in a spacer length-independent manner (Fig. 1), that is, all hybrids produced

stronger anti-epitope antibody responses than did epitope-only peptides. We have shown previously that coupling a MHC class II epitope through a simple polymethylene spacer to an Ii-Key motif enhances substantially the potency of CD4+ T cells activation both in vitro and in vivo [2–4]. In those studies, the enhancement of CD4+ Th cell activation is spacer length-dependent, with the most potent hybrid being the one in which Ii-Key links to the P1 residue of the epitope through one *ava*. A mechanism for the phenomenon of space length-dependent enhancement of T cell activation and spacer length-independent enhancement of antibody production by Ii-Key is proposed below. B cell activation needs help from antigen-specific Th cells through both T-B cell contact and cytokine release. For the activation of Th cells, hybrids are picked up by dendritic cells (DC) through cell surface MHC class II molecules. The direct charging of hybrids to MHC class II molecules on DC may be spacer length-dependent and thus the activation of epitope-specific Th cells exhibits a spacer length-dependency [2–4]. In order to produce epitope-specific antibodies, epitopes or hybrids must first be picked up by B cells through surface IgD and internalized for binding to MHC class II molecules, which then travel to the surface for Th cell recognition. The Ii-Key group may greatly facilitate the binding of a hybrid to MHC class II molecules inside of the B cell under acidic conditions in a spacer length-independent fashion.

We detected only gp100(46–58)-specific IgG1 antibody, but not IgG2a when IFA of CFA were used as adjuvants. Our observations are consistent with those of others [6,7] who have shown that IgG1 dominates the antigen-specific antibody response when IFA or CFA are used. However, our data are not consistent with the results of other's [8] who have found that CFA induced both antigen-specific IgG2a and IgG1 antibodies. In our studies, gp100(46–58)-specific IgG2a titers were evident only when vaccinations were performed using CpG (Fig. 1). The fact that 4 out of 10 mice in hybrid E group and 2 out of 10 mice in peptide A group induced higher IgG2a titer indicates that a vaccine boost might be necessary to induce IgG2a in all mice. Our studies indicate that IL-4 production in CFA immunization [in preparation] might inhibit an isotype switch to IgG2a while CpG enhances IgG2a production through down-regulation of IL-4 [9,10]. In previous experiments, immunization with IFA did not induce a T cell response, indicating that IFA only induced a humeral response; this finding is consistent with previous reports.

Our data indicate that Ii-Key the technology is not only a novel method to significantly enhance the potency of peptide vaccines but also provides a novel tool for enhancing epitope-specific antibody production. In addition, these studies indicate that a chemically simple adjuvant (such as CpG) may be better than compound adjuvants, such as CFA, at inducing a strong immune response against epitope peptides such as the Ii-Key/MHC class II hybrid.

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