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Note

Ii-Key/MHC class II epitope hybrid peptide vaccines for HIV

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Abstract

The Ii-Key/MHC class II epitope hybrid acts on MHC class II molecules to facilitate replacement of antigenic peptides with the epitope tethered to the Ii-Key motif. Hybrid peptides linking an immunoregulatory segment of the Ii protein (Ii-Key peptide) through a polymethylene bridge to MHC class II epitopes of HIV gp160 or gag are potent vaccines to elicit $CD4^+$ T cell responses. More potent responses to two MHC class II epitopes, HIV gp160(843–852) or HIV gag(279–292), occurred in mice immunized with Ii-Key hybrid peptides than with epitope-only peptides, as measured in IL-4 and IFN-γ ELISPOT assays of splenic lymphocytes stimulated in vitro by epitope-only peptides. Both the number of responding cells and cytokine output per cell were increased. The Ii-Key/MHC class II epitope hybrid acts on MHC class II molecules to facilitate replacement of antigenic peptides with the epitope tethered to the Ii-Key motif. Such antigenic peptide constructs create opportunities to enhance greatly Th1 or Th2 responses to MHC class II epitopes for therapeutic purposes.

Keywords: MHC class II; Ii protein; Peptide vaccination; Immunodeviation

1. Introduction

Potent MHC class II antigenic peptide vaccines have been created as hybrids joining the Ii-Key segment of the Ii protein through a simple chemical linker to MHC class II epitope peptides. The design of such hybrid vaccine peptides comes from insight into the mechanism for Ii-Key motif within the Ii protein, in regulating antigenic peptide binding into the antigenic peptide binding groove of MHC class II molecules.

The normal process of MHC class II antigen charging and presentation to CD4+ T helper cells is highly controlled. At synthesis, the antigen binding site of MHC class II molecules is blocked by the Ii protein. After the MHC class II molecule/Ii protein complex is transported to a specialized, post-Golgi compartment, the Ii protein is digested away, allowing access to charging by internalized and processed antigenic peptide fragments. The peptide-charged MHC Class II molecules are transported to the cell surface for presentation to CD4+ T cells.

In studying the role of fragments of the Ii protein generated during the staged cleavage and release of Ii during antigenic peptide binding to MHC class II molecules, we identified a sensitive, late cleavage site in part by engineering mutants not cleaved at that site [1,2]. A peptide of 17 amino acids encompassing this site (murine Ii(77–92) sequence: LRMKLPKSAKPVQMR; termed "Ii-Key") was discovered to enhance greatly presentation of I–E-restricted antigenic peptides to responding T hybridomas [3]. Nested C-terminal deletions of Ii-Key showed maximal activity in the N-terminal Ac-LRMKLPK-NH_2, with half-maximal activity being retained with Ac-LRMK-NH_2 [4]. The mechanism was explored further by assaying binding or release of biotinylated hMBP(90–102) from purified exomembranal human MHC class II molecules [5]. Acting through an allosteric site on HLA-DR1 molecules, Ii-Key regulates the binding of hMBP(90–102) peptide to HLA-DR1 molecules.

Linking an Ii-Key core motif LRMK or LRMKLPK through a simple flexible spacer to the antigenic epitope of PGCC(95–104) enhanced the potency of presentation of the antigenic epitope in vitro 200–500 times that of the epitope-alone peptide [6]. In separate experiments, linkage of Ii-Key to an Her2/neu epitope significantly enhances the in vitro stimulation of PBMC from healthy individuals and breast cancer patients [7].

In order to evaluate the in vivo activity of Ii-Key hybrids, we have designed a series of hybrid peptides

Abbreviations: IFN-γ, interferon gamma; Ii, invariant chain protein; Ii-Key, a peptide from Ii protein: Ii(77–92) and shorter homologs

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comprising Ii-Key core motifs LRMK and the antigenic epitope of HIV gp160(843–852) and HIV gag(279–290). Here, we have shown that linkage of Ii-Key to either HIV gp160(843–852) or HIV gag(279–290) significantly enhances the activity of such hybrid vaccines.

2. Materials and methods

2.1. Design and synthesis of Ii-Key/MHC class II epitope hybrids

Ii-Key hybrids were constructed with the MHC class II epitope (YRAITHIPR) of the well-studied MHC class II-presented peptide HIV gp160(843–852) [8] (Table 1) with high scoring H-2E^k and H-2E^b binding motifs (http://syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/EpPredict.htm). The N-terminus of that epitope was linked to Ac-LRMK by no spacer (gp160-A), by one -*ava*- (gp160-B), or by two -*ava*- (gp160-C), and immunogenecity of these constructs was compared with the epitope-only peptide (gp160-D). The flexible polymethylene spacer 5-aminopentanoic acid (δ-aminovaleric acid: -*ava*-) was a non-natural amino acid incorporated during peptide synthesis. The peptides were terminally acetylated and amidated to inhibit exopeptidases. The algorithm-predicted gag epitope gag(282–290) (GenBank: AF355337) was incorporated into a one -*ava*- hybrid (gag-A). The same core gag epitope with N-terminal extension PVS or C-terminal extension EP was incorporated in one -*ava*- hybrids (gag-B) and (gag-C), with (gag-D) being the respective extended epitope-only control peptide.

2.2. Immunizations

B10.A(5R) (H-2E^{k-d}), C3H/HeJ (H-2^k), (C3 × D2)F1/J (H-2$^{k/d}$), and C57/BL6J (H-2^b) mice from Jackson laboratories were immunized subcutaneously at the base of the tail with indicated doses of Ii-Key/HIV MHC class II hybrid or epitope-only peptides dissolved in saline and emulsified with an equal volume of IFA. On day 14, the mice were boosted with the same dose of immunogen. On day 36, the mice were boosted one more time with the same dose of hybrid or peptide, respectively in saline, either intravenously or subcutaneously at the base of the tail. On day 40, splenocytes suspensions were obtained for ELISPOT assays.

Table 1
Ii-Key/HIV gp160(843–852) or HIV gag(279–290) hybrids

Peptide	Amino acid position	Sequence
Gp160-A	843–852	Ac-LRMK-AYRAIRHIPR-NH_2
Gp160-B	844–852	Ac-LRMK-*ava*-YRAIRHIPR-NH_2
Gp160-C	843–852	Ac-LRMK-*ava*-*ava*-AYRAIRHIPR-NH_2
Gp160-D	843–852	Ac-AYRAIRHIPR-NH_2
Gag-A	282–290	Ac-LRMK-*ava*-ILDIKQGPK-NH_2
Gag-B	279–292	Ac-LRMK-*ava*-PVSILDIKQGPKEP-NH_2
Gag-C	282–292	Ac-LRMK-*ava*-ILDIKQGPKEP-NH_2
Gag-D	279–292	Ac-PVSILDIKQGPKEP-NH_2

2.3. ELISPOT assays

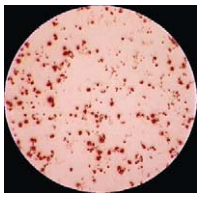
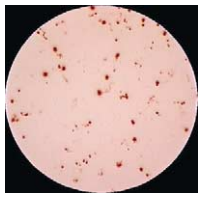
Splenocytes were stimulated in culture for 5 days with 5 μg per well epitope-only peptides. IL-4 and IFN-γ ELISPOT assays were completed with ImmunoSpot M200 plates (Cellular Technology Ltd., Cleveland, OH) according to the methods of Yip et al. [9] and the manufacturer's instructions, using capture antibodies to IFN-γ (4 μg/ml) or IL-4 (2 μg/ml), and indicator antibodies biotinylated anti-mouse IFN-γ (2 μg/ml) or IL-4 (2 μg/ml) (Pharmingen). Digitized images of spots were analyzed with a series 1 Immunospot Analyzer and Immunospot 1.7e software (Cellular Technology Ltd., Cleveland, OH). Criteria for spot size, circularity and color density were determined by comparing control and experimental wells. Partially overlapping spots were separated and noise signal caused by substrate precipitation and non-specific antibody binding was eliminated. Only areas meeting the specified criteria were counted as spots. Mean and standard deviations were calculated for all assays. Total spot size area was the product of mean area times number of cells.

3. Results

3.1. Activity of the HIV gp160(843–852) hybrids

Immunogenecity of HIV gp160-A, -B, and -C hybrids and HIVgp160-D epitope-only peptide was tested in C3H/HeJ (H-2^k), C57BL/6 (H-2^b), and B10.A(5R) (H-2E^{k-d}) mice. The HIV gp160-A and -C hybrids elicited little or no immune responses in all three mouse strains (data not shown). In B10.A(5R) mice there was a consistently greater IL-4 in vitro recall response to the HIV gp160-D epitope-only peptide in mice immunized with 60 nM of HIV gp160-B hybrid than with gp160-D epitope-only peptide (Table 2). Mean spot numbers and S.D. averaged from three mice per group in four to six wells are reported. Comparable results were obtained in two additional experiments without statistically significant differences. However, the HIV gp160-B hybrid did not induce potent immune responses in C3H/HeJ or C57/BL6 mice. An ELISPOT response of 200 spots/10^6 splenocytes is excellent, relative to peptide immunization studies of others [9,10], indicating Ii-Key enhances the immune response against epitope-only peptide. There was little or no IFN-γ response to these hybrids, and that pattern was not changed by use of IFA or CFA. Others have reported in peptide immunizations preferred Th1 induction with CFA and Th2 induction with IFA [9]. This dichotomy is not rigid, and our studies further showed that these adju-

Table 2
HIV gp160 MHC class II epitope hybrid immunization

	In vivo immunogen		
	gp160-B[a]	gp160-D[b]	Naive[c]
			
Spots: in vitro stimulator			
gp160-D	208 ± 78	52 6 ± 6 14	12 ± 10
None	6 ± 6	17 6 ± 6 23	4 ± 1

[a] Injection of immunogen in IFA subcutaneously at the base of the tail on days 0, 14, and 36. On day 40, single splenocyte cell suspensions were restimulated in cultures (10^6 cells per well) containing peptide (5 μg per well) and ELISPOT assayed for IL-4 or IFN-γ cytokine recall responses. Data are expressed as the mean and S.D. spot forming cells from three mice tested individually in one experiment representative of three performed. Deviation in S.D. reflects mouse to mouse variability.

[b] The activity of gp160-D was the positive control for epitope-only peptide immunogenicity. Activity of hybrids was compared with epitope-only peptide.

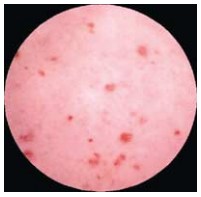
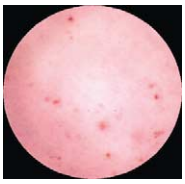
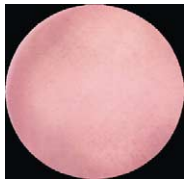
[c] Positive controls for the ELISPOT included naive splenocytes (3×10^5 cells per well) stimulated with PMA (50 ng/ml) and ionomycin (1 μg/ml) generated a mean number greater than 200 spots per well.

vants did not selectively alter the apparent Th1 versus Th2 polarity of the immune response to each epitope.

3.2. Activity of HIV gag(279–292) hybrids

The activities of HIV gag-A, -B, and -C hybrids and HIV gag epitope-peptide only (gag-D) were tested in C3H/HeJ (H-2^k) and (C3 × D2)F_1/J (H-$2E^{k/d}$) mice. Since gag-A induced minor IFN-γ but no IL-4 responses in both C3H/HeJ and (C3 × D2)F_1/J mice (data not shown), two additional HIV/gag hybrids, gag-B and gag-C with N- and C-terminal extensions, were designed to enhance immunogenicity and tested in C3H/HeJ mice (Table 1). Overall, the gag-B hybrid was more active than the gag-C hybrid. It induced three times more IFN-γ responding cells than did the Gag-D epitope-only peptide (Table 3). Furthermore, the total spot size of the gag-B hybrid response was greater than gag-D epitope (Table 3). Spot morphology reflects kinetics and net per cell IFN-γ output as a function of signal strength and duration [11]. The hybrid structure appears to induce a stronger signal associated not only with higher frequency of cytokine producing cells, but also greater cytokine output per cell. Overall, the gag-hybrids consistently elicited IFN-γ responses even with either IFA or CFA, while gp160 hybrids elicited IL-4 responses.

Table 3
HIV gag MHC class II epitope Ii-Key hybrid immunizations

	In vivo immunogen		
	Gag-B[a]	Gag-D[b]	None[c]
			
Spots: in vitro stimulator			
Gag-D	35 ± 15	13 ± 8	0
None	3 ± 3	2 ± 2	0
Total spot size	3.5 ± 1.8	0.8 ± 0.48	0

[a] Injection of immunogen in IFA subcutaneously at the base of the tail on days 0, 14, and 36. On day 40, single splenocyte cell suspensions were restimulated in cultures (10^6 cells per well) containing peptide-epitope only (5 μg per well) and ELISPOT assayed for IL-4 or IFN-γ cytokine recall responses. Data are representative of two experiments performed with similar results.

[b] The activity of gag-D here was the positive control for epitope-only peptide immunogenicity. Activity of hybrids was compared with epitope-only peptide.

[c] Positive controls for the ELISPOT included naive splenocytes (3×10^5 cells per well) stimulated with PMA (50 ng/ml) and ionomycin (1 μ/ml) generated a mean number greater than 200 spots per well.

4. Discussion

Coupling a MHC class II antigenic epitope through a simple polymethylene bridge to an Ii-Key motif increases substantially the potency of presentation to T cells in vitro [6] and, here, in vivo. The Ii-Key peptide regulates the ease of peptide release/charging at the antigenic peptide-binding site of MHC class II molecules [3–5]. Finding a bridge length of one -*ava*- to be more potent than two -*ava*- is consistent with the view that within a hybrid, the Ii-Key moiety binds initially to an allosteric site just outside the trough formed by two anti-parallel alpha helices, but it is pulled from that site as the antigenic epitope settles into a functional conformation in the antigenic peptide-binding site. One -*ava*- (2.5 peptidyl backbone units when extended) is too short to bridge the distance in DR4/Ii CLIP peptide crystallographic models from the P1 site to the region where LRMK is hypothesized to lay [12].

Comparable effects of N-terminal amino acids were seen in long members of a homologous series of CLIP peptides in lability of binding to MHC class II molecules [13]. Likewise, similar binding-lability influences were seen in hybrids between N-terminal CLIP sequences and antigenic epitopes, but without the simple polymethylene spacer used here [12].

Although the Ii-Key-recognized allosteric site on MHC class II molecules is available on the surface of living or formaldehyde-fixed cells [4], the expression of the motif XOXOX where X is a member of the chemically equivalent group leucine, isoleucine, valine, phenylalanine, methionine and O is another amino acid suppressed below an empirical distribution at $P < 0.001$ [14]. That is, there are few naturally occurring peptidyl fragments capable of acting at that site on cell surface MHC class II molecules to spill endogenously bound epitopes, and the Ii-Key motif is destroyed during Ii cleavage from MHC class II molecules [1,15].

Dominance of IL-4 response over IFN-γ with HIV gp160-B hybrid and dominance of IFN-γ over IL-4 with gag-B hybrid indicates a trend of Th2 and Th1 responses, respectively. Such a conclusion could be strengthened by concurrent analysis of IL-4 and IL-10 cytokines. Similar immunodeviation of responses with respect to sequence-modified epitope-only peptides occur by several mechanisms, including peptide dose, affinity and time of maturation [16,17]. Future studies can characterize the mechanisms underlying these responses. Relevant to predicting a robust clinical benefit of Ii-Key hybrids, we also demonstrated that hybrids elicited higher frequencies of responding cells and stronger cytokine output per cell, in a manner paralleling studies of the effects of signal strength in eliciting effector functions [11]. T helper cells acting on B cells through a presented MHC class II epitope, augment their responses to surface antibody-recognized protein antigens. Likewise, T helper cells augment CTL activity by stimulating dendritic cells to become more potent activators of CTL.

Insertion of MHC class II epitopes into an Ii-Key hybrid peptide so increases vaccine potency, that many more diagnostic and therapeutic uses become feasible, than were possible with epitope only vaccine peptides. For example Ii-Key/HER-2/neu peptides are significantly more sensitive in measuring in vitro responses of patients with breast cancer [7]. Such hybrid peptides should be preferred to immunize breast cancer patients [18,19]. Likewise, Ii-Key/melanoma gp100 and Ii-Key/tyrosinase can be constructed with peptides already used clinically to treat some melanoma patients [20–22]. The enhancement of T helper activation will contribute to more potent responses to either endogenously presented or synthetic CTL epitopes [23–25].

Ii-Key/Fel d 1 hybrid peptides provide a more potent, and perhaps more safely delivered, immunodeviating response in patients with allergy to cat dander [26]. One advantage of the Ii-Key hybrids method of immunodeviation is that the epitope itself is not altered. Synthesizing the Ii-Key-spacer motif into combinatorial libraries of putative MHC class II epitopes will enhance identification of epitopes in such libraries.

In the case of viral infections, the enhanced sensitivity afforded by Ii-Key hybrids in measuring T cell responses could be invaluable in early diagnosis of viral infections, such as the SARS agent or respiratory syncitial virus. Here, we have demonstrated the sensitivity of Ii-Key/HIV MHC class II epitope hybrids. Such hybrids can also be used to vaccinate individuals, either as a stand-alone vaccine, or in preparation for a DNA vaccine, or before an attenuated virus vaccine. For example, in the case of vaccinia inoculations to protect against smallpox, complications of the Dryvax® preparation has lead to a ring vaccination strategy in which only potential first responders in a population are vaccinated [27]. Mass vaccination is not pursued. However, adequate mass vaccination with Ii-Key hybrids containing an appropriate baskets of MHC class II epitopes might decrease the frequency of complications upon administering the Dryvax® preparation, or the death rate for individuals contracting smallpox with other protective vaccine.

Acknowledgements

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