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ORIGINAL ARTICLE

Decision-making critical amino acids: role in designing peptide vaccines for eliciting Th1 and Th2 immune response

Khurram Mushtaq · Sathi Babu Chodisetti · Pradeep K. Rai · Sudeep K. Maurya · Mohammed Amir · Javaid A. Sheikh · Javed N. Agrewala

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Abstract CD4 T cells play a cardinal role in orchestrating immune system. Differentiation of CD4 T cells to Th1 and Th2 effector subsets depends on multiple factors such as relative intensity of interactions between T cell receptor with peptide-major histocompatibility complex, cytokine milieu, antigen dose, and costimulatory molecules. Literature supports the critical role of peptide's binding affinity to Human Leukocyte Antigens (HLAs) and in the differentiation of naïve CD4 T cells to Th1 and Th2 subsets. However, there exists no definite report addressing very precisely the correlation between physicochemical properties (hydrophobicity, hydrophilicity), pattern, position of amino acids in peptide and their role in skewing immune response towards Th1 and Th2 cells. This may play a significant role in designing peptide vaccines. Hence in the present study, we have evaluated the relationship between amino acid pattern and their influence in differentiation of Th1 and Th2 cells. We have used a data set of 320 peptides, whose role has been already established experimentally in the generation of either Th1 or Th2 immune response. Further, characterization was done based on binding affinity, promiscuity, amino acid pattern and binding conformation of peptides. We have observed that distinct amino acids in peptides elicit either Th1 or Th2 immunity. Consequently, this study signifies that alteration in the sequence and type of selected amino acids in the HLA class II binding peptides can modulate the differentiation of Th1 and Th2 cells. Therefore, this study may have an important implication in providing a platform for designing peptide-based vaccine candidates that can trigger desired Th1 or Th2 response.

Keywords Binding affinity · Multiple sequence alignment · Promiscuous peptides · Th1 and Th2 immune response

Abbreviations

HLA	Human leukocyte antigen
MSA	Multiple sequence alignment
pTh1 and pTh2	Peptides eliciting Th1 and Th2 immune response, respectively

Introduction

CD4 T cells play a central role in eliciting and balancing the immune response against invading pathogens. These cells identify antigenic peptides through T cell receptor (TCR) in the context of major histocompatibility complex (MHC) expressed on the surface of antigen-presenting cells (APCs). Interaction of peptide–MHC complex (p-MHC) with TCR and triggering through co-stimulatory molecules lead to the optimum activation of naïve CD4 T cells (Zhu and Paul 2008). CD4 T cells can be subdivided into Th1, Th2, Th17 and Treg subsets (Dubey et al. 1995). Differentiation of naïve CD4 T cells into a particular subset depends on multiple factors like the strength of p-MHC-TCR interaction, delivery and nature of co-stimulatory signals and cytokines milieu (Murray 1998; Constant and Bottomly 1997). Th1 cells mainly produce IFN-γ and

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K. Mushtaq · S. B. Chodisetti · P. K. Rai · S. K. Maurya · M. Amir · J. A. Sheikh · J. N. Agrewala (✉)
Immunology Laboratory, CSIR-Institute of Microbial Technology, Chandigarh 160036, India
e-mail: javed@imtech.res.in

TNF-α, and are responsible for cell-mediated immunity. These cells are vital in the elimination of intracellular pathogens like *Leishmania major*, *Mycobacterium tuberculosis*, *Human immunodeficiency virus*, etc. (Reiner and Locksley 1995; North and Jung 2004). On the other hand, Th2 cell chiefly secretes IL-4 and IL-5 (Mosmann and Coffman 1989). Th2 cells are responsible for allergic reactions and provide immunity against extra-cellular pathogens like helminthes such as *Nippostrongylus brasiliensis*, *Schistosoma mansoni*, etc. (Pulendran and Artis 2012).

Presence of IL-12 and IL-4 drives naïve CD4 T cells to Th1 and Th2 subsets, respectively (Swain et al. 1990). Apart from the cytokines, other factors may also selectively control the development of Th1 and Th2 cells. It has been reported that p–MHC interactions also specifically influence the differentiation of naïve CD4 T cell to either Th1 or Th2 cells (Kumar et al. 1995). Nevertheless, there is a strong correlation between peptide affinity to TCR and the type of immune response elicited. The affinity and specificity of a particular peptide to its TCR are mainly determined only by specific amino acid residues called anchoring residues. Peptides with strong affinity to TCR induce more Th1 response, whereas those with low affinity prompt Th2 response (Kumar et al. 1995; Iezzi et al. 1999; Boyton and Altmann 2002; Badou et al. 2001). Interestingly, some peptides have been reported to possess intrinsic property to induce Th1 or Th2 response (Whelan et al. 2000; Zhang et al. 2001). Literature suggests that proteins may have intrinsic motifs which may selectively induce Th1 or Th2 response (Guy et al. 2005; Nakajima-Adachi et al. 2012). The influence of altering the sequence of the peptide on elicitation of Th1 and Th2 immune response has been previously described (Kumar et al. 1995; Pfeiffer et al. 1995; Soloway et al. 1991; Liew et al. 1990; Milich et al. 1995). These studies indicated that the peptide–MHC interaction can control not only the magnitude, but also the direction of the functional immune response. Apart from this peptide-MHC interaction, strength of signaling through TCR ligand interaction can also regulate this lineage development (Constant and Bottomly 1997; Iezzi et al. 1999; Boyton and Altmann 2002; Blander et al. 2000). Recently, it has been even shown that strength of T cell stimulation is a vital factor that determines the ability of T cells to become IL-17 producers (Purvis et al. 2010). Moreover, single amino acid-substituted alternate peptide ligands (APL) or antagonists can induce immune deviation in T cell populations, indicating that cytokine profiles can be altered by stimulation with ligands of reduced affinity (Pfeiffer et al. 1995).

Peptides thus alone can determine the type of Th1 and Th2 response (Whelan et al. 2000; Zhang et al. 2001); however, so far no study has been conducted to find the type and position of amino acids in the peptide epitope, which can bias Th1 or Th2 response. Understanding the relationship between amino acids pattern and type of immunity elicited may provide a platform for designing a desired Th1 or Th2 epitope-based vaccine. Hence, in the present study, we employed in silico methods to identify amino acid and pattern in the peptides responsible for skewing immune response towards Th1 or Th2 lineage. We have selected set of peptides inducing either Th1 or Th2 response experimentally. We identified certain conserved anchoring residues responsible for inducing Th1 and Th2 immune response. Strikingly, there were considerable differences in the binding affinity and conformation of these peptides. This finding may have an important application in designing novel peptide vaccine candidates for generating selective Th1 or Th2 immunity.

Materials and methods

Peptide data set

A total data set of 320 peptides was retrieved from Immune epitope database (IEDB), website: http://www.iedb.org (Vita et al. 2010). These peptides were selected based on the ability to induce IFN-γ (for Th1 response) or IL-4 (for Th2 response). This data set included peptide from distinct origins like virus, bacteria, protozoan, allergens, and auto antigens and showed diverse HLA restriction (HLA DR, DP and DQ).

Selected HLA-DR alleles

Human leukocyte antigen class II (HLA-II) alleles predominant in human population, i.e., DRB1_0101, DRB1_0301, DRB1_0401, DRB1_0405, DRB1_0701, DRB1_1101, DRB1_1302, DRB1_1501, and DRB5_0101 were selected for the study.

Programs and databases

IEDB

This database contains sequences related to T cell epitopes, immunogens, allergens, autoantigens and alloantigens. It also provides data of peptide binding to diverse HLA alleles (Vita et al. 2010).

ProPred

ProPred is a prediction server that uses matrix-based pocket profiles to determine the binding region of peptide to HLA class II alleles (Singh and Raghava 2001). It also predicts the promiscuous binding regions in antigenic peptides.

SVMHC

This server uses matrix-based method for prediction of HLA class II binders. It predicts binding regions within a peptide and also provides information about HLA alleles anchoring residues (Donnes and Elofsson 2002).

NetMHC2.2

NetMHC2.2 server predicts binding of peptides to various HLA class II alleles using artificial neural networks (ANNs). It also calculates binding affinity of peptides to a particular HLA class II allele (Nielsen et al. 2007).

ClustalW

ClustalW is a multiple sequence alignment program used for clustering protein sequences. It uses neighbor joining method as a default method (Larkin et al. 2007).

Classification and characterization of peptides inducing Th1 and Th2 response

It has been already reported that peptides bind to HLA class II molecules with a common amino acid pattern (Rothbard and Taylor 1988). To identify various peptide patterns, we have selectively chosen a set of peptides which are experimentally proven to be inducers of either IFN-γ or IL-4, which are the hallmark cytokines for Th1 and Th2 response, respectively. Henceforth, such peptides that induce Th1 and Th2 response will be referred to as 'pTh1' and 'pTh2', respectively, in the text. Within the selected peptides, binding regions were predicted using ProPred and SVMHC prediction servers which have relatively high accuracy (Gowthaman and Agrewala 2008; Wang et al. 2008). We have considered consensus binding by combining the results of these two servers and classified peptides as binders and non-binders. The binding affinity of these peptides to a particular HLA class II allele was calculated using NetMHC 2.2 server based on IC_{50} values. These were classified as strong binders ($IC_{50} \leq 50nM$) and weak binders ($50nM \leq IC_{50} \leq 500nM$). The peptides having IC_{50} value of >500nM were considered as non-binders. Peptides exhibiting binding to 3 or more than 3 of selected alleles were considered as promiscuous peptides. Multiple sequence alignment of binding regions restricted to particular HLA allele was performed using ClustalW to identify patterns and conserved amino acid sequences. The results were then analyzed using Jalview considering the binding affinity of peptides to HLA alleles, type of anchoring residues and nature of amino acids present in binding region (Waterhouse et al. 2009).

Molecular docking of model peptides with MHC

Peptide sequences known to induce Th1 or Th2 response were selected from literature (Agrewala and Wilkinson 1998). The initial peptide structure was generated using "build and edit protein" program of Accelrys Discovery studio (Accelrys software Inc. 2007). The conformations were then submitted to energy minimization followed by molecular dynamics simulation for 20 nano seconds in an implicit solvent model using GROMACS (Hess et al. 2008). The refined peptide conformations were used as starting ligand molecule and docked with HLA-DR2 crystal structure using AutoDock (Huey et al. 2007). For this, we have chosen crystal structure of HLA-DR2 bounded with a peptide from human myelin basic protein (PDBID: 1BX2). The existing peptide in PDBID: 1BX2 was deleted and docking studies were performed with p91–110 and p21–40 peptides. Out of the ten binding poses predicted by AutoDock, we selected optimal binding pose based on the binding free energy. Results were analyzed using AutoDock Tools (Sanner 1999) and PyMol (DeLano 2002).

Results

pTh1 are strong binders and binding affinity increases with addition of flanking residues

pTh1 and pTh2 were analyzed for their distinct amino acid patterns responsible for promiscuous nature and binding affinity to selected HLA alleles using bioinformatics tools as described in methods (Fig. 1; Table S1). Peptides were classified as binders and non-binders based on binding affinity to HLA alleles (Fig. S1). As promiscuity is one of the vital attributes of peptide vaccines, binders were further classified as promiscuous and non-promiscuous pTh1 and pTh2, based on their ability to bind with three or more HLA alleles. We observed almost same ratio of promiscuous and non-promiscuous peptides in both pTh1 and pTh2 (Fig. S2A). Promiscuous peptides were checked for binding affinity towards their respective alleles. We observed that most of the promiscuous pTh1 and pTh2 were strong binders (Fig. S2B). However, strong binding affinity was associated with 20 mer pTh1 as well as pTh2 (Figs. S3, S4). These results reveal that there was a strong correlation between length of peptide and their binding affinity. To establish correlation between peptide length and binding affinity, IC_{50} values of peptides with varying length were compared. Even though, overall length of each peptide was variable but the binding region consisted mainly of nine amino acids. Taking 20 mer peptides out of the data set as model peptides, we analyzed the trend in

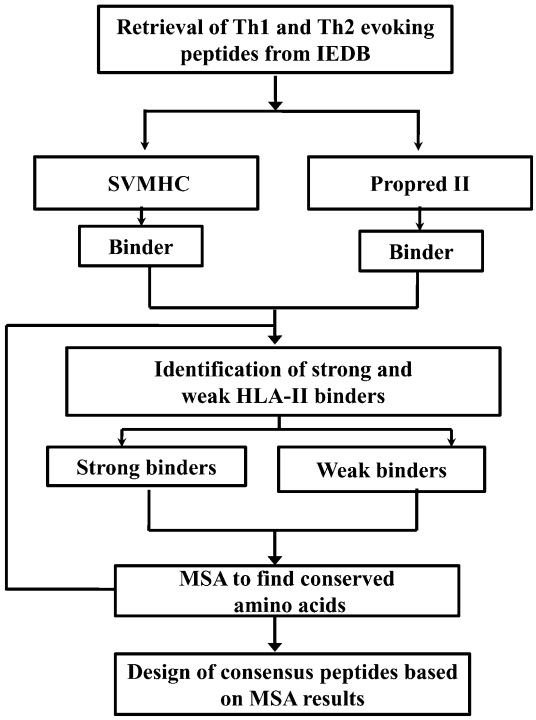

Fig. 1 *Characterization of Th1 and Th2 cells skewing peptides and their amino acid binding sequences to HLA alleles*. Th1 and Th2 cells activating peptides were selected and classified as binders and non-binders to HLA alleles by SVMHC and ProPred-II. Binders were further identified as strong and weak binders by NetMHC2.2. The anchor residues were determined and multiple sequence alignment (*MSA*) was performed to find out pattern of conserved amino acids as strong and weak HLA binders and also checked the permissive binding of each peptide

binding affinity and peptide length by varying the length of adjoining amino acids around 9 mer binding core. Interestingly, we noticed that binding affinity improved considerably by increasing the length of the peptide (Fig. 2a, b). Although the affinity was strong with the length of 15 amino acids, there was further improvement on sequential addition of the flanking residues. Moreover, we noticed that binding affinity of the core region was much higher in case of pTh1 as compared to pTh2 and the same trend was observed with increasing the length of the peptide. The pTh1 peptides exhibited higher binding affinity than pTh2 (Fig. 2a, b). In addition, in the case of pTh1 strong binders were more whereas for pTh2 weak binders were abundant (Fig. 2c).

Predominance of hydrophobic amino acids in the peptide at the position 4 and 5 skews immune response towards Th1 and Th2, respectively

We performed MSA to identify conserved amino acids in total pTh1 and pTh2. Both pTh1 and pTh2 had conserved hydrophobic amino acids at position 1. However, there were more aliphatic amino acids in case of pTh1 and aromatic amino acids for pTh2 peptides (Fig. 3a, b). We also observed that hydrophobic amino acids were predominantly present at positions 3 and 5 in the case of pTh2 peptides but not in pTh1. pTh1 showed the presence of 100 % hydrophobic amino acids at position 4, in contrast to pTh2, that displayed conserved hydrophobic residue at position 5. Moreover, the same trend of hydrophobicity was observed with additional HLA-DR alleles, i.e., DRB1_0102, DRB1_0305, DRB1_0402, DRB1_0405, DRB1_0805, DRB1_1301 and DRB1_1502 (Fig. S5). Further, at the position 4, leucine was the most abundant and conserved hydrophobic amino acid for pTh1 (Fig. 4). There was almost equal distribution of hydrophobic and hydrophilic amino acids at positions 7 and 9 for both pTh1 and pTh2 (Fig. 3a, b). These results indicate that positions 3, 4 and 5 are crucial for designing peptides that may tune immune response towards Th1 and Th2 cells. Next, we calculated the consensus sequences of both pTh1 and pTh2 strong binders with respect to the selected HLA alleles and checked their binding affinity. We observed very strong affinity of resultant consensus sequences for all pTh1 (Table 1a). On the contrary, pTh2 consensus sequences failed to express strong uniform binding affinity (Table 1b). The data may support the idea that these consensus sequences could be used as a template to design synthetic peptides vaccine to elicit desired Th1 and Th2 immune response.

We also investigated amino acid pattern responsible for promiscuous nature of peptides by multiple sequence alignment (MSA). It was observed that the charged amino acids were conserved at position 3 in case of promiscuous pTh1. In contrast, hydrophobic amino acids were predominantly present at position 3 in case of non-promiscuous pTh1 peptides (Fig. 5a, b). In case of promiscuous pTh2, hydrophobic amino acids were primarily occupied at position 3 and 4; whereas these positions were occupied by hydrophilic amino acids in non-promiscuous pTh2 (Fig. 5c, d). These results indicate that there is a conserved amino acid pattern in pTh1 and pTh2, which ultimately dictates the promiscuous nature and binding strength of a peptide to a particular HLA allele.

Molecular docking reveals conformational differences in pTh1 and pTh2-MHC complex

To gain insights into the effect of different amino acids in pTh1 and pTh2 on skewing the resultant immune response, we checked for spatial conformation of peptides inside the MHC class II binding groove using molecular docking. For this, peptides already known to skew immune response towards either Th1 (p91–110) or Th2 (p21–40) were used (Agrewala and Wilkinson 1998). A lower binding free energy indicates a more stable protein–ligand system. It was observed that both peptides p91–110 and p21–40

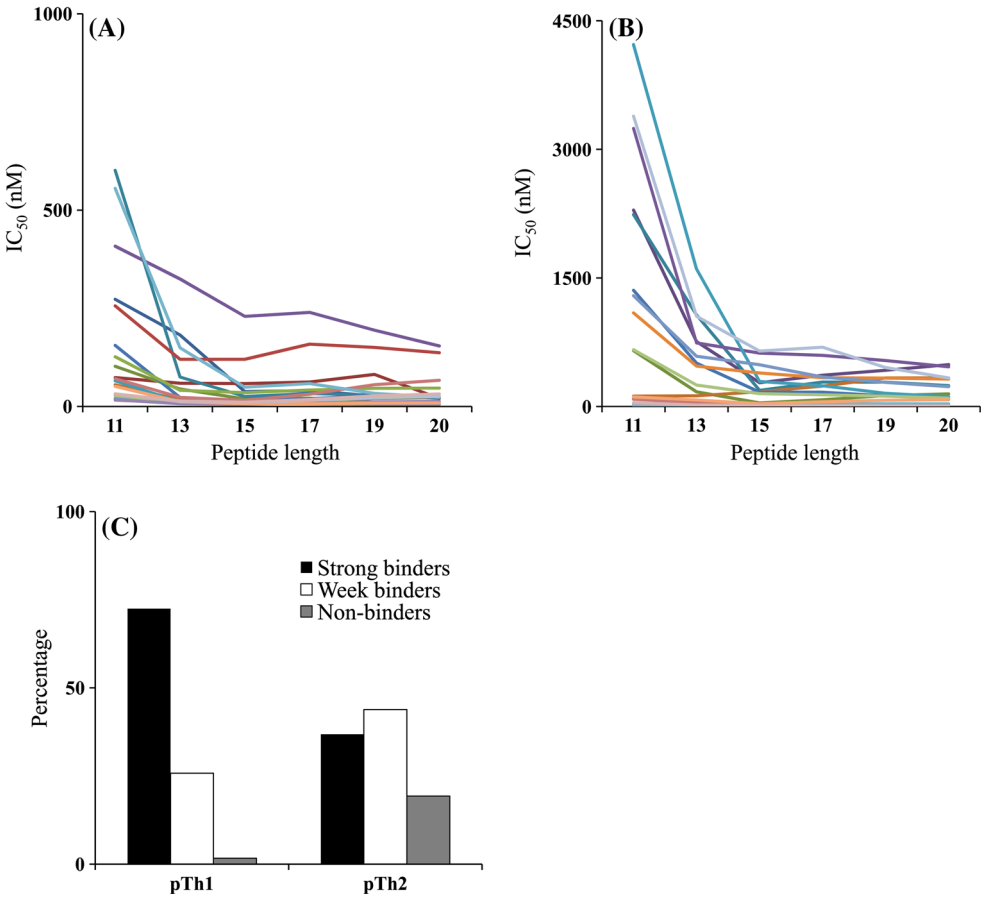

Fig. 2 *Relation between length of peptide and binding affinity. Line plot* represents change in IC_{50} value with increase in length of the pTh1 (**a**) and pTh2 (**b**), respectively. *Bar diagram* represents the percentage of strong and weak binders used in the study. *X*-axis represents the number of amino acids in each peptide, whereas *Y*-axis indicates IC_{50} value

showed higher binding affinity towards HLA molecule with values of 10.85 and 12.87 nM, respectively. However, both peptides acquired different conformations inside the MHC binding groove. It was also noticed that peptide p91–110 acquired a linear conformation inside the MHC binding groove as compared to p21–40 (Fig. 6a, b). Also both the peptides differed in terms of non-covalent interactions with the MHC binding groove, as p91–110 showed more number of hydrogen bonds. In case of p91–110, amino acid residues participating in non-covalent interactions were uniformly distributed over the length of the peptide. However, in case of p21–40, non-covalent interactions were limited to terminal region of the peptide (Fig. 6c, d). In addition, the same observation has been found with peptides selected from pTh1, pTh2 data set and their consensus sequences (Fig. S6 A, B). Over all, these results designate significant difference in the binding conformation of pTh1 and pTh2 peptides.

Discussion

T helper cells mediate adaptive immunity against several infectious agents. Th1 and Th2 cells cross regulate the function of each other (de Montmollin et al. 2009). In general, immune response to whole antigen is attributed by the presence of both Th1 and Th2 epitopes (Basu et al. 2005). Hence, epitope-based vaccines have potential advantage of selecting immunodominant epitopes that can incline immune response towards Th1 or Th2 cells. Further, undesired autoreactive and immunosuppressive pathogenic epitopes can be eliminated. Thus, a profound understanding of underlying mechanisms of peptide and MHC allele interactions can provide a skeleton to develop synthetic peptides which can be attractive vaccine candidates.

Many factors can influence the differentiation of naïve CD4 cells into a Th1 or Th2 cell, and these factors need not be mutually exclusive. There may well be other dominant influences but the impact of epitope specificity on Th phenotype development should not be underestimated. The affinity of the p-MHC-TCR interaction is thought to be an essential factor for determining the differentiation of CD4 T cells into Th1 or Th2 phenotypes.

In the present study, we have tried to establish a correlation between amino acid pattern in a specified peptide and elicitation of either Th1 or Th2 response. Following major findings have emerged from the study: (1) majority

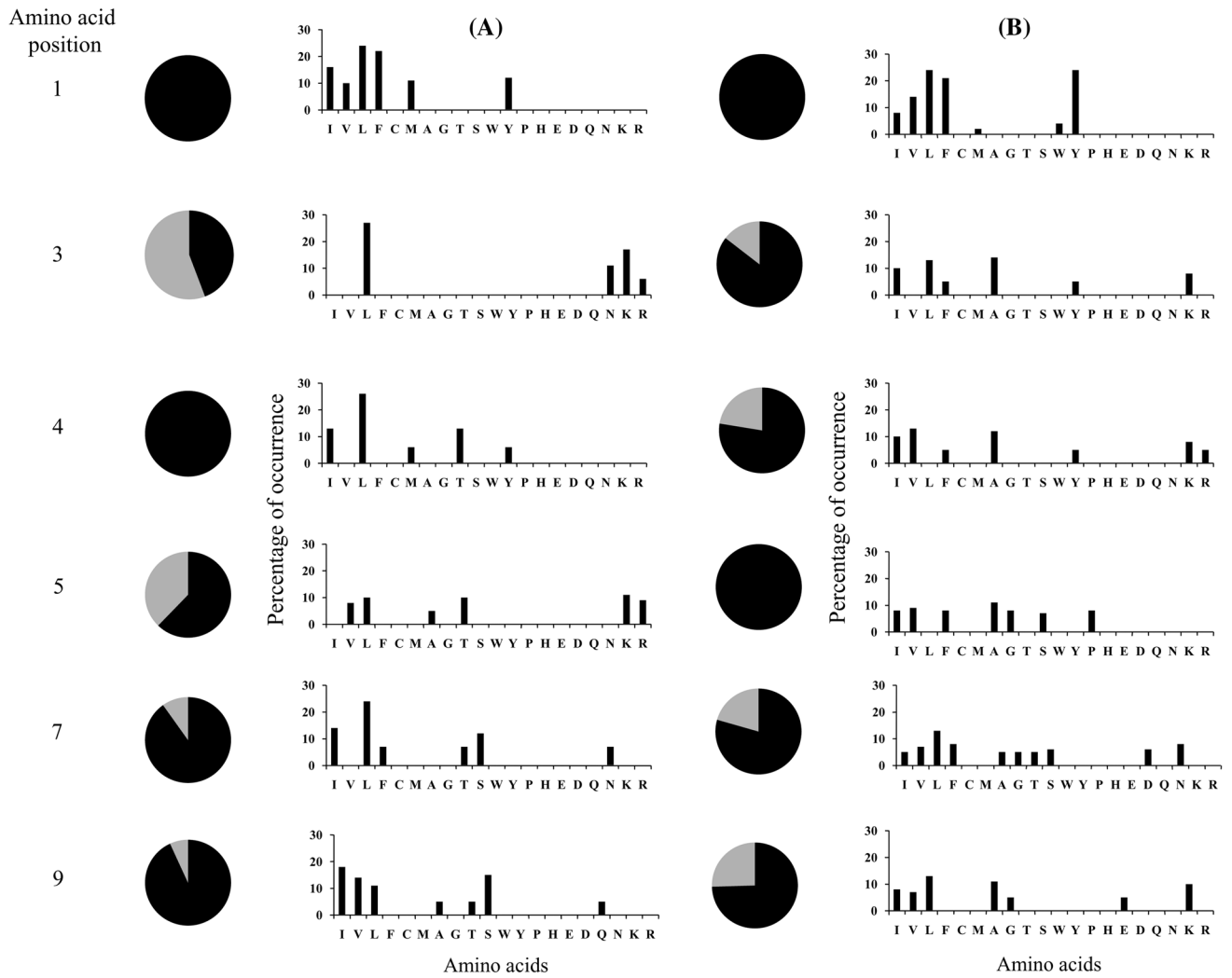

Fig. 3 *Position of amino acids in the MHC binding groove is distinct for inducing Th1 and Th2 response*. *Pie diagrams* represent percentage of conserved hydrophobicity (*black*) and hydrophilicity (*gray*) at each position of peptide-binding region that evoke Th1 (**a**); Th2 (**b**) response. Each *bar* graph represents percentage of amino acid at the indicated position of binding region. *X*-axis represents the hydrophilicity in an increasing order and *Y*-axis percentage of amino acid at that particular position

of the pTh1 were strong binders, whereas pTh2 were weak binders; (2) hydrophobic amino acids were conserved at position 1 for both pTh1 and pTh2; (3) position 3 of promiscuous pTh1 was predominantly occupied by charged amino acids whereas by hydrophobic amino acids in pTh2; (4) 100 % hydrophobicity was observed at position 4 of pTh1, whereas at position 5 of pTh2; (5) at position 4, leucine was the most predominant hydrophobic amino acid for pTh1 but absent in pTh2; (6) nature of amino acids at positions 3, 4, 5 was very crucial for distinct Th1 and Th2 responses; (7) there were conformational differences in the binding nature of pTh1 and pTh2 to MHC molecules.

Although all the peptides have equal length of binding region (9 amino acid), strong affinity was associated with peptides spanning 20 amino acids in case of both pTh1 and pTh2. These results were further supported by several reports, which showed the importance of peptide flanking

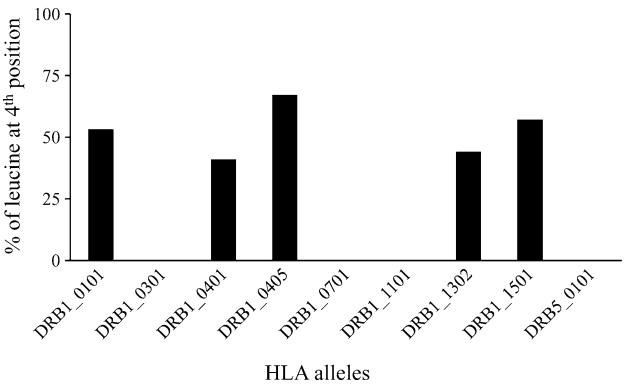

Fig. 4 *Leucine is highly conserved at the fourth position in Th1 eliciting peptides, irrespective of its binding to various HLA alleles. Bar diagram* represents percentage of leucine residue at the fourth position of HLA-binding peptides evoking predominantly Th1 response. Multiple sequence alignment of peptides was performed to check the sequence conservation of leucine with respect to various HLA alleles

Table 1 Consensus sequences derived from multiple sequence alignment of strong binders to a particular HLA allele

	HLA allele	Binder/non-binder	IC_{50} value
(a) Thl consensus sequence			
SKNKEGLQAGLKLLGTNSLSTEWSLCSVT	DRB1_0101	Strong binder	5.8
QAKYLQKIQNILSTEWSPCSLT	DRB1_0401	Strong binder	26.0
EYLKKIQNSLSSELSPLSVLE	DRB1_0405	Strong binder	37.5
QAGFFLGlTKRILSGEWIPLSLD Al.F	DRB1_0701	Strong binder	15.7
QQAGFFLLTGISLTIPQSLDKL	DRB1_1101	Strong binder	36.8
DVENIVKEISAKIITPCL	DRB1_1302	Strong binder	6.1
NRRGKIIVGIYGNGVVAL	DRB1_1501	Strong binder	16.5
VGGVYILLRRGPRlGVR	DRB5_0I01	Strong binder	8.0
(b) Th2 consensus sequence			
ASGGASASFALAAVAAAAGSK	DRB1_0101	Strong binder	5.3
GPFKYEKDRVDVFDH	DRB1_0401	Non-binder	602.6
VHFFAAAVTNPAT	DRB1_0405	Strong binder	8
LRAVESNLLYV	DRB1_0701	Strong binder	18.3
YFAMLIKLPAGTK	DRB1_1101	Strong binder	6.1
No strong binders were detected	DRB1_1302	–	–
IPAFRYVKAFTLEV	DRB1_1501	Strong binder	16.5
TPFKYVFSVFDEVPH	DRB5_0101	Weak binder	214.4

The data represent list of consensus sequences of Th1 (a); Th2 (b) inducing strong binders, derived from multiple sequence alignment using Jalview. It also represents respective alleles, and their binding nature in terms of IC_{50} values

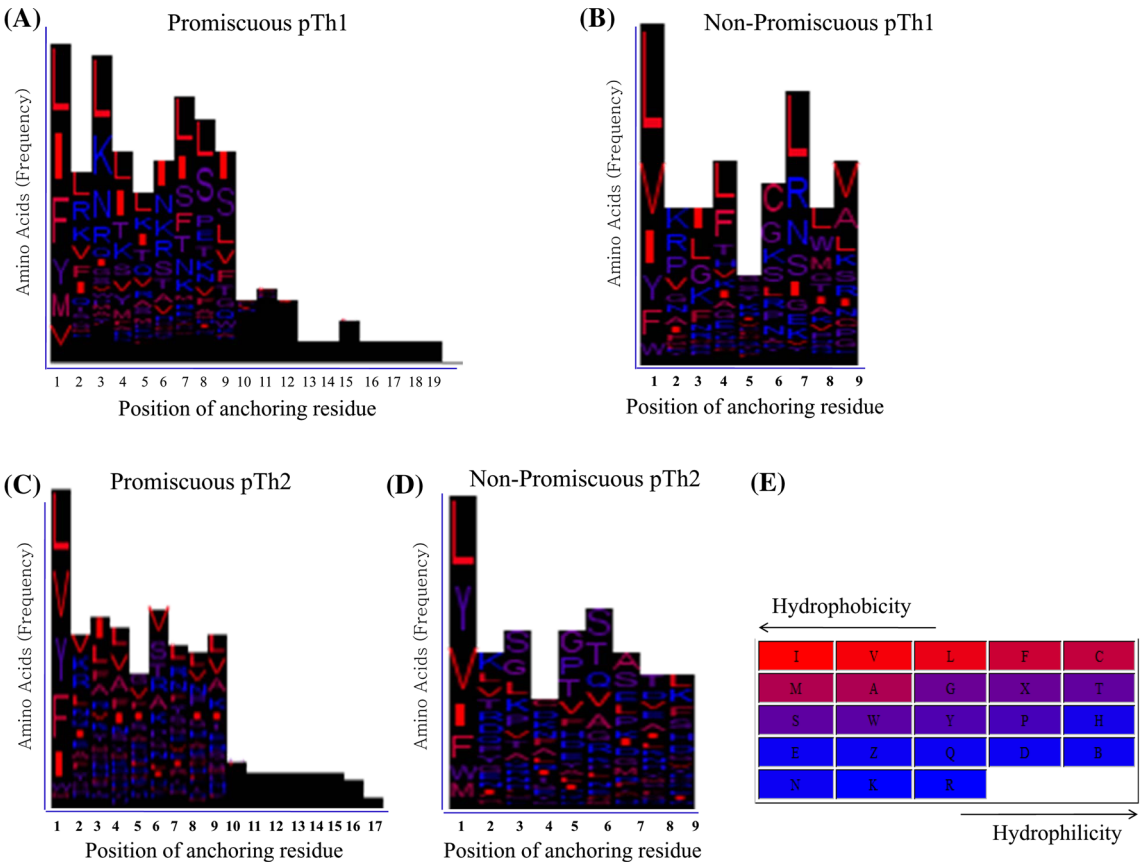

Fig. 5 *Multiple sequence alignment reveals the hydrophobicity and hydrophilicity of Th1 and Th2 eliciting peptides.* Consensus logo corresponds to promiscuous peptides evoking Th1 response (**a**); non-promiscuous Th1 peptides (**b**); promiscuous Th2 peptides (**c**); non-promiscuous Th2 peptides (**d**). The amino acid residues are colored according to hydrophobicity based on Kyte and Doolittle scale (**e**). *Red* represents most hydrophobic and *blue* most hydrophilic amino acids. *X*-axis depicts the position of binding of amino acids of a peptide to HLA molecule and *Y*-axis frequency of a particular amino acid (color figure online)

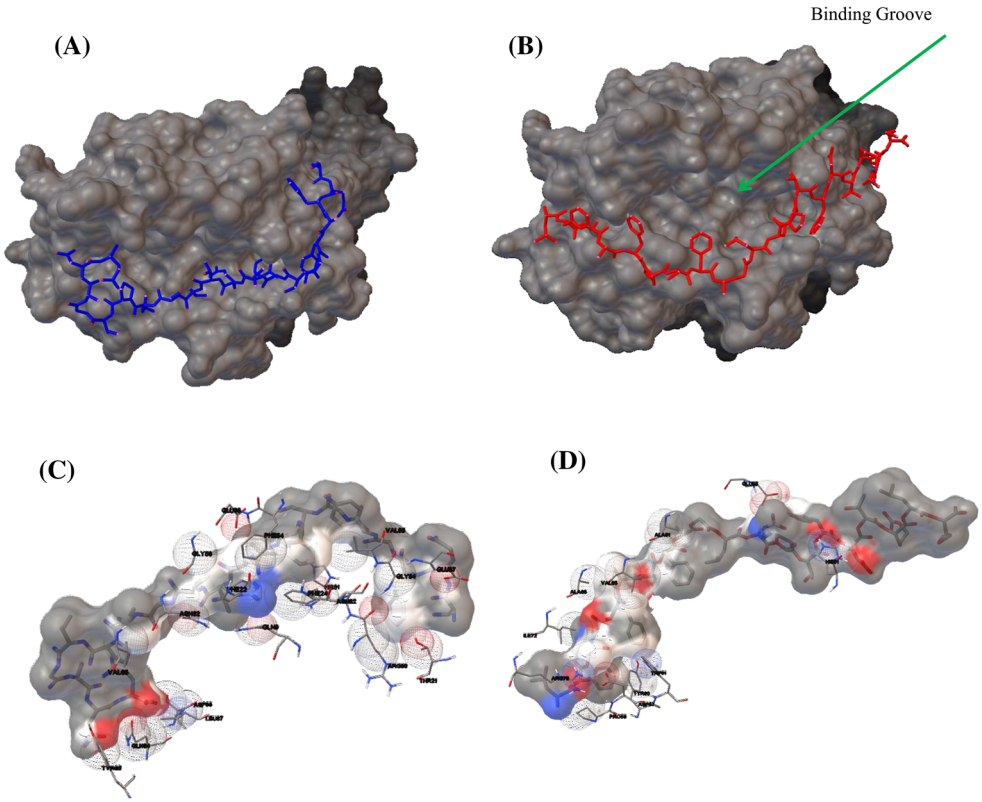

Fig. 6 *Difference in conformational binding of Th1 and Th2 inducing peptides.* Figure represents conformations of p91–110 (**a**) and p21–40 (**b**) docked with HLA-DR2 molecule. The HLA molecule is shown in surface representation and peptides as stick representation. **c**, **d** Represent non-covalent interactions of p91–110 and p21–40 respectively, with the HLA binding groove

residues (PFRs) present outside the MHC anchor region in determining the binding affinity (Arnold et al. 2002; O'Brien et al. 2008). Although the peptide data set has been experimentally validated and in most cases by overlapping peptide libraries, exclusively identifying the true ligands that would have been isolated from the cells after peptide dissociation from its cognate MHC would be preferable. Still we employed the in silico analysis to predict the binder nonamer peptide out of the sequence and analyze its binding efficiency with sequential addition of flanking amino acid residues. This approach of computational prediction has been shown to be in harmony with experimental data (Zhang et al. 2012; Tongchusak et al. 2008; Pereira et al. 2011) and apart from some obvious advantages needs some refinement to overcome the limitations (Brusic et al. 2004; Lafuente and Reche 2009; Liao and Arthur 2011).

Further, we investigated amino acids responsible for promiscuity of peptides. Notably, we have observed that position 3 is critical for promiscuous binding of both pTh1 and pTh2. In case of pTh1, promiscuity was associated with occurrence of charged/polar amino acids, whereas non-promiscuity was attributed to hydrophobic amino acids present at position 3. In contrast, reverse order was observed in the case of pTh2. This observation signifies that the nature and sequence of amino acids in the peptide vaccine can determine the Th1 and Th2 immunity. It is well established that different antigens have tendency to specifically trigger either Th1 or Th2 response (Whelan et al. 2000; Zhang et al. 2001). Hydrophobic amino acids present at positions 1, 7 and 9 were found to be conserved in both Th1 and Th2 eliciting peptides, indicating importance of these positions in MHC–peptide binding, irrespective of type of immune response. However, positions 3, 4 and 5 were differentially conserved in pTh1 and pTh2. This observation was further validated with additional HLA alleles, indicating that these positions may have important bearing in designing a peptide vaccine to specifically elicit Th1 or Th2 response.

The present study also denotes that a specific amino acid pattern can favor immune response towards either Th1 or Th2 immunity. Further, it establishes a strong correlation between nature, position and binding affinity of the amino acids that ultimately dictates the desired immune response. Hence a particular position of an amino acid may be quite crucial in a peptide to differentially regulate the Th1/Th2 immunity. Furthermore, our study designates that manipulation of the position and the type of amino acids in a peptide vaccine can bias immune response towards Th1 and avoiding the Th2 lineage, or vice versa. It is important to mention here that Th1 and Th2 reciprocally regulate the generation and function of each other (de Montmollin et al. 2009). Therefore, the presence of one phenotype may impair the function of other, hereby rendering vaccine

ineffective. We suggest that it is very important to carefully choose the physicochemical properties and position of amino acids, while designing a peptide vaccine. Further, in a peptide vaccine, amino acids and their patterns should be mapped to identify their role in Th1 or Th2 response. The nature of majority of antigens/peptides derived from pathogens or host tissues still largely remains unidentified for their vaccination potential for eliciting Th1 or Th2 immunity. In this regards, our current study contributes to provide information in successfully designing a vaccine employing in silico methods. This information will ultimately help in profitably synthesizing a peptide for mass vaccination in a cost-effective manner (Black et al. 2010).

In essence, the knowledge about position, properties and binding affinity of amino acids provided in the current study will be extremely beneficial in designing peptide-based vaccines, which may have potential in taming and tuning the immune system to evoke either Th1 or Th2 response to combat and eliminate intracellular and extracellular pathogens, respectively.

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Conflict of interest The authors have no financial conflict of interest.

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