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SCIENCE DIRECT
Vaccine 24 (2006) 2225–2229
Vaccine
www.elsevier.com/locate/vaccine

Short communication

Peptide-binding assays and HLA II transgenic Aβ° mice are consistent and complementary tools for identifying HLA II-restricted peptides

Stéphane Depil [a,b,1], Gerhild Angyalosi [a,1], Olivier Moralès [a], Myriam Delacre [a], Nadira Delhem [a], Violaine François [a], Bertrand Georges [a], Juergen Hammer [c], Bernard Maillère [d], Claude Auriault [a], Véronique Pancré [a,*]

[a] *UMR 8527 CNRS/Lille II/Institut Pasteur, Institut de Biologie, Lille, France*
[b] *EFS Nord de France, Lille, France*
[c] *Research Informatics, Genetics and Genomics, Hoffmann-La Roche Inc., Nutley, New Jersey, USA*
[d] *Département d'Ingénierie et d'Etude des Protéines, CEA-Saclay, Gif sur Yvette, France*

Received 19 April 2005; received in revised form 4 November 2005; accepted 24 November 2005
Available online 9 December 2005

Abstract

The identification of MHC class II-restricted peptides has become a priority for the development of peptide-based prophylactic and therapeutic vaccines. The aim of this study was to assess the correlations between peptide-binding assays on purified HLA II molecules and immunization of human HLA II transgenic mice deficient in murine class II molecules (Aβ°). We used as models two MHC class II-restricted peptides, one derived from the HIV Nef regulatory protein (Nef $_{56–68}$) and the other from the *Schistosoma mansoni* 28-kDa glutathione-S-transferase (Sm28GST $_{190–211}$). High correlations were found between the two approaches, which showed that the Nef $_{56–68}$ and Sm28GST $_{190–211}$ peptides may represent promiscuous ligands for HLA-DQ and for HLA-DR molecules, respectively. We suggest a rational method based on the combination of peptide-binding assays and HLA II transgenic mice experiments as consistent and complementary tools for selecting T helper epitopes.

Keywords: MHC class II; Peptide; Binding assays; Transgenic mice; Vaccine

1. Introduction

Several strategies have been proposed for identifying MHC class II-restricted epitopes. An empirical method consists of testing in vitro the $CD4^+$ T-cell responses induced by a panel of overlapping peptides which cover the protein of interest, then selecting the peptides against which reactivity is observed [1–3]. Another strategy uses T-cell epitope prediction programs in order to define peptides potentially capable of binding multiple HLA II molecules [4–7]. In this context, in vitro class II binding assays have been developed to confirm the affinity of the selected peptides for different HLA II molecules [7,8]. Transgenic mice expressing human MHC class II molecules also provide a useful model for identifying HLA II-restricted epitopes [9–14]. It has been shown that the coexpression of endogenous murine H-2 class II molecules reduces the intensity of human HLA II-restricted antigen-specific responses in HLA II transgenic mice, by favoring murine over human MHC recognition and education [15]. This prompted us to use HLA II transgenic mice deficient in H-2 class II expression (Aβ°). Combining class II binding assays and HLA II transgenic Aβ° mice allowed us to confirm the HLA restriction patterns of two different peptides, one derived from the HIV Nef regulatory protein and the other from the *Schistosoma mansoni* 28-kDa glutathione-S-transferase (Sm28GST). We show here that these two approaches represent consistent and complementary techniques.

* Corresponding author. Tel.: +33 3 20 87 12 42; fax: +33 3 20 87 10 19.
E-mail address: Veronique.Pancre@ibl.fr (V. Pancré).
[1] SD and GA equally participated to this work.

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doi:10.1016/j.vaccine.2005.11.048

2. Materials and methods

2.1. Peptides

Nef $_{56–68}$ (AWLEAQEEEEVGF) and Sm28GST $_{190–211}$ (ENLLASSPRLAKYLSNRPATPF) peptides were synthesized on an Advanced Chemtech model 357 MPS Synthesizer (Advanced Chemtech Europ, Brussels, Belgium) as previously described [16]. Homogeneity was confirmed by analytical HPLC.

2.2. HLA class II peptide-binding assays

EBV homozygous cell lines were used as source of human HLA class II molecules [17]. As previously described [8], purified HLA-DR and HLA-DQ molecules were incubated with a referenced biotinylated peptide in the presence of serial dilutions of Nef $_{56–68}$ or Sm28GST $_{190–211}$ competitor peptide. Data are expressed as the peptide concentration that prevented binding of 50% of the labeled peptide (IC_{50}).

2.3. HLA transgenic Aβ° mice

C57BL/6 mice expressing different HLA II alleles (DR2, DR3, DQ6 and DQ8) and deficient in murine class II molecules (Aβ°) were a kind gift of Dr. Ch. David (Mayo Clinic Rocheester, MI, USA) [18]. Mice expressing the HLA-DR1 transgene on an FVB/N background were kindly provided by Dr. D. Altmann (Hammersmith Hospital, London, UK) [19] and backcrossed with C57BL/6 Aβ° mice [20]. HLA transgenic Aβ° mice were immunized s.c. with the Nef $_{56–68}$ or the Sm28GST $_{190–211}$ peptide (50 μg) in CFA (Sigma–Aldrich, Saint Quentin Fallavier, France) and two booster injections with peptide (25 μg) in IFA (Sigma–Aldrich) at 2 weekly intervals were performed.

2.4. Analysis of proliferative response

The proliferative response was measured as previously described [21] by incubating 5×10^5 splenic or lymph node cells, removed 7 days after the last injection, with an optimal concentration of peptide (25 μg) for 5 days. The cell culture supernatants were tested for cytokine release.

2.5. Antibody and cytokine detection

The quantification was performed by ELISA as previously described [13,21]. Mouse sera were diluted 1/100 for total IgG, IgG1 and IgG3 and 1/10 for IgG2a and IgG2b detection and peroxydase labeled anti-mouse IgG1 (dilution 1/3000) or IgG2a dilution 1/2000) were provided by Diagnostic Pasteur (Marnes-la-Coquette, France). IFN-γ release in the supernatants was detected using sandwich ELISA. The antibody pair used for the detection was provided by BD PharMingen (San Diego, CA, USA). Absorbances at 492 nm were measured using a multichannel spectrophotometer (Titertek Multiskan MCC 1340). Results were expressed as the mean of duplicate wells after substraction of the background.

3. Results

3.1. Prediction of HLA-DR-restricted epitopes

We used TEPITOPE, a T-cell epitope prediction program for HLA-DR molecules [4], to analyze the sequence of the Nef $_{56–68}$ and Sm28GST $_{190–211}$ peptides. The Sm28GST $_{190–211}$ peptide was predicted to contain promiscuous binding motifs for HLA-DR. In particular, several potential epitopes binding to the HLA-DR1, HLA-DR2 and HLA-DR3 molecules (which correspond to the HLA-DR transgenic Aβ° mice used in this study) were identified. In contrast, no HLA-DR binding motif was found inside the Nef $_{56–68}$ peptide (data not shown).

3.2. High binding capacity of the Nef $_{56–68}$ peptide to HLA-DQ alleles and of the Sm28GST $_{190–211}$ peptide to HLA-DR alleles

The two peptides were submitted to binding assays specific for HLA-DR and HLA-DQ alleles frequently encountered in the Caucasian population (Table 1). It has been reported that 1000 nM IC_{50} may be defined as the affinity threshold associated with immunogenicity in the context of HLA-DR molecules [22]. The results were also expressed in terms of relative activity. This latter is the ratio between

Table 1
Binding capacities of the Sm28GST $_{190–211}$ peptide to HLA class II molecules

	Alleles	Allelic frequencies (%)	Referenced peptide	Referenced peptide IC_{50} (nM)	Sm28GST$_{190–211}$ IC_{50} (nM)	Relative activity
DR1	DRB1*0101	9.3	$HA_{306–318}$	5.5	80	14.5
DR2	DRB1*1501	8.0	$A3_{152–166}$	3	120	40
DR3	DRB1*0301	10.9	$MT_{2–16}$	125	150	1.2
DR4	DRB1*0401	5.6	$HA_{306–318}$	30	200	6.7
DR7	DRB1*0701	14.0	YKL	125	2000	16
DR11	DRB1*1101	9.2	$HA_{306–318}$	23	225	9.8
DR13	DRB1*1301	6.0	$B1_{21–36}$	1000	1250	1.25
DQ6	DQA1*0103/DQB1*0603	6.2/5.8	$B7_{150–164}$	2750	>10000	>36.4
DQ8	DQA1*0301/DQB1*0302	14.2/8.1	$DQB_{45–57}$	200	1500	7.5