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Research paper

A simple competitive assay to determine peptide affinity for HLA class II molecules: A useful tool for epitope prediction

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ABSTRACT

We have designed a cytometry-based competition assay to evaluate peptide binding to empty recombinant HLA class II molecules. The efficiency of this assay was evaluated using recombinant HLA-DP0401 molecules (HLA-DP) produced in insect cells and 13 peptides from human telomerase reverse transcriptase (hTERT). We demonstrate that our method allowed accurate measurements of peptide Ki values and can thus discriminate strong, moderate and poor HLA-DP binders. In parallel, we showed that among hTERT peptides, the most immunodominant in healthy individuals were those with moderate affinity for HLA-DP while no T cell response could be evidenced against peptides with very strong or very low affinities for HLA-DP. This strongly suggests that the precise determination of peptide affinity with our method can improve HLA class II epitope prediction.

1. Introduction

It is now well accepted that vaccination strategies with peptides in infectious diseases and cancer should activate both CD4 and CD8 responses to be efficient (Wang and Livingstone, 2003; Kobayashi and Celis, 2008; Melief and van der Burg, 2008). Therefore, there is a great need to identify immunodominant CD4 epitopes from viral or tumour antigens in various HLA class II contexts. A number of groups have developed algorithms to predict peptide binding to HLA class II molecules using data bases of already identified epitopes and in some instances, refined their models with experimental in vitro binding data (Rammensee et al., 1999; Bui et al., 2005; Busson et al., 2006; Nielsen et al., 2007). Some of us have previously developed and described a competition binding assay using immunoprecipitated HLA class II complexes from EBV cell lines and a biotinylated reference peptide (Busson et al., 2006). This competition assay proved its efficiency to analyse binding of peptides from the protein survivin or from the MAGE-A family to multiple HLA-DR and DP molecules and to predict CD4 T cell epitopes (Wang et al., 2007; Wang et al., 2008). More recently, Justesen et al. described a method based on the refolding of recombinant α and β chains of MHC class II, separately produced in *E. coli*, in the presence of competing peptides (Justesen et al., 2009). However, this technique remained fairly cumbersome and no data was provided in this publication to document the immunogenicity of the selected peptides.

Our goal was to develop a simple competitive assay on recombinant HLA class II molecules that would provide a Ki value for each peptide and then assess the relevance of this Ki for the prediction of immunogenicity. Indeed, one of the drawbacks of using immunoprecipitated class II molecules in

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a competition assay is that it is difficult to standardize the number of binding sites i.e. the fraction of class II molecules that are in a "peptide-receptive" conformation (Natarajan et al., 1999) since most complexes will already be loaded with peptides of various affinities. Thus, only relative affinities of peptides expressed as IC50 against a chosen reference peptide can be determined.

In the present paper, we describe a cytometry-based competition assay using empty recombinant HLA-DP*0401 molecules (HLA-DPB1*0401 and HLA-DPA*1031 chains) produced in S2 cells and an FITC-labeled NYESO-$1_{157-169}$ reference peptide. We used this assay to measure the affinities of 13 peptides from the protein hTERT (Human telomerase reverse transcriptase) from which many HLA-class I epitopes and a few HLA-class II epitopes have already been described (for review, (Liu et al., 2010)) and compared with the values of IC50 assessed by our previous method. We then evaluated the correlation between Ki values and the capacity of each peptide to generate specific CD4 T cell-lines following in vitro sensitization of peripheral blood CD4 T lymphocytes from healthy DP0401 donors.

2. Material and methods

2.1. Generation of DP0401 expressing S2 cell line and clones

Empty HLA-DP0401 molecules were produced following the protocol described by Yang et al. (Yang et al., 2005). In brief, HLA-DPA1*0103 and DPB1*0401 cDNAs were cloned in frame with leucine-acidic (LZA) and leucine-basic (LZB) sequences respectively in pRmHa3. A cDNA coding for a biotinylation peptide was added to the 3′ end of the DPB1*0401 construct to allow multimerization of the produced protein. Drosophila Schneider 2 (S2) were cultured at 27 °C in Drosophila Serum Free Medium (Invitrogen) supplemented with L-glutamine at 200 mM (Sigma). S2 cells were transfected with expression vectors carrying genes encoding the extracellular domains of HLA-DP0401 α and β chains and with the selection vector pCo-Blast (Invitrogen) using the calcium phosphate transfection kit from Invitrogen (K2780-01). To obtain stable long-term clones, cells were selected for 3 weeks with 25 μg/ml blasticidin (Invitrogen) and then cloned by limiting dilution on autologous feeder cells (irradiated S2 cells). Individual clones were expanded and expression of HLA-DP0401 molecules was induced with 1 mM CuSO4. Five days post-induction, HLA-DP0401 production was tested by intracellular staining of clones.

2.2. Expression, purification and biotinylation of HLA-DP0401 monomer in S2 cells

A S2 cell clone that expressed high level of HLA-DP0401 was expanded in 6 × 500 mL of culture medium in a shaking incubator at 27 °C. Cells (10^7/mL) were then induced with 1 mM of CuSO4 for 5 days. Culture supernatant was clarified by centrifugation and filtered through 0.45 μm filter. An initial purification step of clarification, concentration and capture was then performed with the Streamline expanded bed absorption technology (Streamline Q Sepharose chromatography, GE Healthcare). These anion exchange beads were equilibrated with Tris 20 mM pH 8 Tween 0.005%, loaded with diluted sample (9 L) and washed in suspension bed mode at a linear flow rate. Elution was then performed with Tris 20 mM pH 8 Tween 0.005% NaCl 0.5 M in packed bed mode. An example of SDS PAGE analysis of two eluted fractions from 2 distinct preparations is shown on Fig. 1 panel A. Selected fractions were pooled and loaded onto a 5 mL anti-HLA-DP mAb (B7.21) affinity column (GE Healthcare). The column was equilibrated and washed with Tris 20 mM pH 8 Tween 0.005% NaCl 0.5 M and eluted with a 0.1 M citric buffer pH3 immediately neutralized with 1 M of Tris pH9. As shown on Fig. 1 panel B, gel filtration analysis of the eluted fraction on a S200 Superdex 200 column (24 ml, GE Healthcare) confirmed that a single peak of α/β DP complexes was purified with a retention volume of 13 ml. DP complexes were then biotinylated with BirA enzyme (Avidity, Denver, CO) in biotinylation buffer (50 mM bicine pH8.3, 10 mM ATP, 10 mM magnesium acetate, 50 μm d-biotin) for 5 hours at 30 °C. Excess biotin was removed by

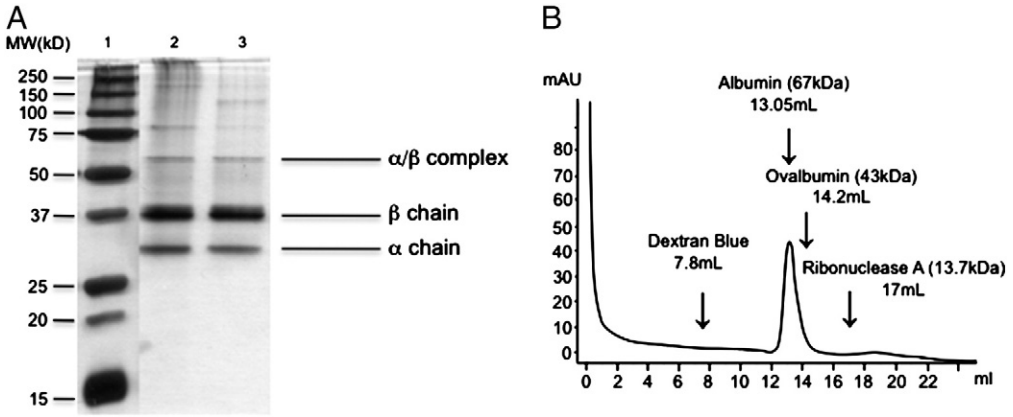

Fig. 1. (A) SDS-Page analysis of HLA-DP0401 molecules. Two distinct elution fractions of DP affinity chromatography column were loaded under non-reducing conditions (lane 2 and 3) and revealed by silver nitrate staining. Molecular weight markers were run in lane 1. (B) Purified HLA-DP0401 monomers analyzed by gel filtration on a Superdex 200 column appeared as a single peak with a retention volume of 13 ml. Standard molecular weight markers are indicated.