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Strong and Specific Protective and Therapeutic Immunity Induced by Single HLA-A2.1 Restricted Epitope DNA Vaccine in Rabbits

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Abstract

An HLA-A2.1 transgenic rabbit /Cottontail rabbit papillomavirus (CRPV) infection model has been reported previously. In this study, we incorporated online MHCI epitope prediction software and HLA-A2.1 transgenic mouse and rabbit systems together to demonstrate an efficient way to identify and test immunogenicity of two HLA-A2.1 restricted epitopes from CRPVE1 (161-169 LLFRQAHSV and 303-311 MLQEKPFQL). Both epitopes were able to induce specific cytotoxic CD8 T cells in HLA-A2.1 mice and rabbits after peptide and DNA immunization and in vitro stimulation respectively. Using an epitope DNA vaccination method, we achieved partial and complete protection against CRPV DNA challenge by CRPVE1/161-169 and CRPVE1/303-311 respectively in HLA-A2.1 transgenic outbred rabbits. CRPVE1/303-311 also showed strong and specific therapeutic effects in CRPV-infected HLA-A2.1 transgenic outbred rabbits. Interestingly, epitope CRPVE1/303-311 (but not E1/161-169) showed strong protective immunity in non-transgenic EIII/JC inbred (but not outbred) NZW rabbits. Our data demonstrates an efficient way to identify HLA-A2.1 restricted epitopes for the development of prophylactic and therapeutic vaccines.

Keywords: Epitope DNA vaccine, A2, transgenic mouse, transgenic rabbit, CRPV

1. Introduction

HLA-A2.1 is a common human MHCI molecule among the Caucasian population. Using an HLA-A2.1 transgenic mouse system, investigators have screened and identified HLA-A2.1 restricted epitopes from a variety of

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pathogens in the past two decades [1]. Online software makes the prediction of a potential HLA-A2.1 restricted epitope possible. Many well characterized epitopes have been tested for therapeutic effects for viral infection or tumor formation in the A2 mouse model [2, 3]. The disadvantage of the HLA-A2.1 mouse model is the limited susceptibility of the mouse to some human pathogens such as HTLV-1 and syphilis. As a result, it is difficult to predict the protective effects of these and other identified epitopes with this model system. In contrast, the rabbit is susceptible to these pathogens and thus provides an excellent host to test the immunogenicity of different targets. Therefore, we established an HLA-A2.1 transgenic rabbit model in an attempt to compensate for the limitations of the HLA-A2.1 transgenic mouse model [4, 5].

Human papillomaviruses are small DNA tumor viruses, some of which induce malignancy in genital, anal, oropharyngeal and also skin tissues. The viruses show species specificity and thus no animal model is available to study HPV infection *in vivo* [6]. In addition, no mouse papillomavirus model has been reported so far. We used the cottontail rabbit papillomavirus (CRPV) / rabbit model that mimics high-risk HPV infections in the human population [7-9]. We have found that CRPV genome can be modified extensively without losing the ability to induce papillomas in rabbits [10]. These unique characteristics make it possible to test the specific immunity induced by an HLA-A2.1 restricted epitope DNA vaccine by embedding the corresponding epitope in the CRPV genome [5].

In previous studies, we used online MHCI epitope prediction software to screen five HLA-A2.1 restricted epitopes from CRPVE1 and to generate a multivalent epitope DNA vaccine [5]. This vaccine provided complete protection against CRPV infection with a single booster immunization [11]. We further observed that two of the five epitopes (CRPVE1/161-169 and CRPVE1/303-311) could stimulate specific CTLs in the HLA-A2.1 transgenic mouse system by direct peptide immunization. In the current study we have shown that these two epitopes provided strong and specific protective immunity against CRPV DNA infection in HLA-A2.1 transgenic rabbits with both outbred and EIII/JC inbred genetic backgrounds. Partial therapeutic immunity was also induced by CRPVE1/303-311 epitope DNA vaccination in the HLA-A2.1 transgenic outbred rabbits. Immunization with the CRPVE1/303-311 peptide failed to stimulate protective immunity in HLA-A2.1 transgenic outbred rabbits, however. Our data demonstrate a direct correlation between these two transgenic animal model systems showing that these systems can be combined to screen and test protective and therapeutic epitope vaccines *in vivo*.

2. Material and Methods

2.1. Peptide immunization in HLA-A2.1 transgenic mice and rabbits

CRPVE1/161-169 (LLFRQAHSV), CRPVE1/303-311(MLQEKPFQL) and HBV core T helper peptide (TPPAYRPPNAPIL) were synthesized in the core facility of Pennsylvania State University College of Medicine. CRPVE1/161-169 and CRPVE1/303-311 were diluted into 1×PBS buffer (4 mg/ml containing 5% DMSO). HBV core T helper peptide was diluted into 1×PBS buffer (5.6 mg/ml containing 5% DMSO). Each peptide was mixed with HBV core T helper peptide and incomplete Freund's adjuvant (IFA) at a 1:1:2 ratio with a homogenizer until the mixture was emulsified [12]. Each mouse was injected with 50 µl of emulsion on both sides of the base of the tail. Two mice were used for each peptide immunization. The mice were immunized twice with 2-week intervals between injections. Spleens were harvested one week after the booster immunization. HLA-A2.1 transgenic and normal rabbits were immunized with 200 µl of emulsion subcutaneously for 4 times at 2 week intervals. One week after the final booster immunization, the rabbits were challenged with viral DNA on four back sites respectively as described previously [5].

2.2. Tetramer binding assay

After two rounds of *in vitro* stimulations, the bulk CTLs from HLA-A2.1 transgenic mice or rabbits were labeled with CD8-FITC and then specific PE conjugated tetramers (synthesized by the tetramer core facility of the National Institute of Health). A two-color flow cytometry analysis was used for detecting specific tetramer binding CD8 T cells at the core facility of Pennsylvania State University College of Medicine[13].

2.3. Intracellular cytokine assay

Bulk mouse CTLs were cultured in triplicate wells of a 96-well plate with 1µM peptide (either E1 peptides or a reference peptide HIVGagP17/77-85) and 1µM Brefeldin A at 37 ^{o}C for 3-4 hours. The cells were then labeled with FITC conjugated anti-mouse CD8 and PE conjugated anti-mouse interferon gamma and analyzed by two-color flow cytometry at the core facility of Pennsylvania State University College of Medicine as described previously [5].