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Peptide-pulsed dendritic cells induce the hepatitis C viral epitope-specific responses of naïve human T cells

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ABSTRACT

Hepatitis C virus (HCV) is a major cause of liver disease. Spontaneous resolution of infection is associated with broad, MHC class I- ($CD8^+$) and class II-restricted ($CD4^+$) T cell responses to multiple viral epitopes. Only 20% of patients clear infection spontaneously, however, most develop chronic disease. The response to chemotherapy varies; therapeutic vaccination offers an additional treatment strategy. To date, therapeutic vaccines have demonstrated only limited success in clinical trials. Vector-mediated vaccination with multi-epitope-expressing DNA constructs provides an improved approach. Highly-conserved, HLA-A2-restricted HCV epitopes and HLA-DRB1-restricted immunogenic consensus sequences (ICS, each composed of multiple overlapping and highly conserved epitopes) were predicted using bioinformatics tools and synthesized as peptides. HLA binding activity was determined in competitive binding assays. Immunogenicity and the ability of each peptide to stimulate naïve human T cell recognition and IFN-γ production were assessed in cultures of total PBMCs and in co-cultures composed of peptide-pulsed dendritic cells (DCs) and purified T lymphocytes, cell populations derived from normal blood donors. Essentially all predicted HLA-A2-restricted epitopes and HLA-DRB1-restricted ICS exhibited HLA binding activity and the ability to elicit immune recognition and IFN-γ production by naïve human T cells. The ability of DCs pulsed with these highly-conserved HLA-A2- and -DRB1-restricted peptides to induce naïve human T cell reactivity and IFN-γ production *ex vivo* demonstrates the potential efficacy of a multi-epitope-based HCV vaccine targeted to dendritic cells.

1. Introduction

Hepatitis C virus (HCV), a small single-stranded RNA virus, constitutes a major cause of liver disease [1]. The positive-sense genome encodes an ~3000 amino acid poly-protein precursor, which is cleaved by cellular and viral proteases to yield three structural [core, envelope 1 (E1) and E2], and seven nonstructural (p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b) proteins [2]. Spontaneous resolution of HCV infections is associated with broad, MHC class I- ($CD8^+$) and class II-restricted ($CD4^+$) T cell responses to multiple viral epitopes derived from these proteins [3,4]. Unfortunately, only 20% of patients clear infection spontaneously, most develop chronic disease [5]. Seventy to eighty percent of patients infected with HCV genotype 1 (the principal causative agent of hepatitis C in the U.S.) experience a sustained virologic response (SVR) following treatment that includes protease inhibitors, *i.e.*, telaprevir or boceprevir, administered in conjunction with PEGylated interferon and ribavirin. A significant number of those treated remains infected, however, the cost of treatment is high, and the risk and severity of side effects are considerable [6,7]. New approaches to treating chronic HCV infections are urgently needed.

Therapeutic vaccination concurrent with or without drug therapy offers an additional approach to treating chronic hepatitis C. Indeed, the capacity of a significant percentage of patients to resolve acute infections spontaneously suggests that an effective therapeutic vaccine is a realistic goal. A safe and effective vaccine must elicit broad, vigorous $CD4^+$ and $CD8^+$ T cells responses to

Abbreviations: DCs, dendritic cells; HCV, hepatitis C virus; ICS, immunogenic consensus sequences; NS, nonstructural; PBMC, peripheral blood mononuclear cell; SVR, sustained virologic response.

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conserved viral epitopes, which culminate in the elimination of HCV without causing liver pathology. Development of such a vaccine has proven problematic, however, due primarily to: infidelity of the viral RNA polymerase (NS5b), genetic diversity and the rapid emergence of viral variants [8]. To date, a number of vaccine strategies have demonstrated negligible or only limited success in clinical trials [9,10].

Vaccination with HCV epitope expressing dendritic cells (DCs) offers a vector-mediated approach to treating chronic, HCV infected patients. DCs play a central role in $CD4^+$ and $CD8^+$ T cell activation and the induction of immunity [11]. The potential effectiveness of DC-based vaccines in treating chronic hepatitis C has been demonstrated in animal models [12–14]. Moreover, in a recent Phase I clinical trial, chronically-infected patients vaccinated with monocyte-derived DCs pulsed with 6 HCV-specific, HLA-class I-restricted peptides exhibited peptide-specific CD8 T cell responses [15]. These responses were not sustained, however, and there was no effect on viral load suggesting that HCV clearance might require vaccination with DCs that expressed a broader range of viral epitopes. Toward this end, immunoinformatics tools were used to predict 21 HLA-A*0201-restricted epitopes and 19 HLA-DRB1-restricted immunogenic consensus sequences (ICS, each composed of multiple epitopes), which were highly-conserved and encoded by HCV genotype 1. These predicted epitopes/ICS were synthesized as peptides and their capacities to bind HLA molecules were determined. Subsequently, their immunogenicity and ability to elicit the peptide-specific responses of naïve human T cells were validated in an *in vitro* peripheral blood mononuclear cell (PBMC) immunogenicity assay. Similarly, monocyte-derived DCs pulsed with these same peptides induced the epitope-specific responses of naïve $CD4^+$ and $CD8^+$ T cells in culture demonstrating the potential efficacy of a multi-epitope-based HCV vaccine that targets dendritic cells.

2. Materials and methods

2.1. Genome collection

HCV polyprotein sequences marked complete, representing 516 genotype 1a and 355 genotype 1b isolates, were acquired from the Los Alamos sequence and immunology database [16,17].

2.2. Conserved 9-mer search

Nine-mer amino acid sequences, constituting the length of the peptide chain that fits into the binding groove of the HLA class I and class II molecules, were parsed out of the total 871 polyproteins and compared for identical parsed 9-mers in matching open reading fames of other genotype 1a or 1b isolates using the Conservatrix algorithm [18]. The potential immunogenicity of these identical 9-mer sequences was predicted using the computational method described below.

2.3. Epitope mapping

Approximately 50% of the U.S. population expresses cell-surface HLA-A2; essentially the entire population expresses one or more HLA-DRB1 alleles [19,20]. Consequently, each 9-mer was scored for its predicted potential to bind a panel of eight HLA-DRB1 alleles (DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0801, DRB1*1101, DRB1*1301 and DRB1*1501) using EpiMatrix, a matrix-based algorithm for mapping T cell epitopes [21,22]. Additionally, all parsed 9-mers were scored for the potential to bind HLA-A2. Putative HLA-A2 epitopes were selected based on conservation in genotype 1a and b, EpiMatrix HLA A2-matrix predicted binding score, and reports of the *ex vivo* response of PBMCs obtained from HCV-infected patients.

2.4. Immunogenic consensus sequences

HLA-DRB1-restricted ICS were constructed using EpiAssembler, an algorithm that maximizes epitope density by assembling potentially immunogenic 9-mers (identified using EpiMatrix) into 18–25 amino acid stretches [23]. To avoid potential cross-reactivity with the human proteome, any peptide that shared more than 7 identities per 9-mer frame was eliminated from further consideration [24]. The final HLA-A2- and -DRB1-restricted peptide sequences were synthesized using FMOC chemistry and purified >85% by HPLC (21st Century Biochemicals, Marlboro, MA).

2.5. HLA binding assay

The capacity of predicted epitopes (peptides) to bind multiple HLA-DRB1 alleles was assessed using a competitive, HLA class II binding assay as we described previously [24,25], using HLA molecules obtained from Bill Kwok, Benaroya Research Institute, Seattle, WA). Assays were performed for HLA-DRB1*0101, -DRB1*0301, -DRB1*0401, -DRB1*0701, -DRB1*1101 and -DRB1*1501, alleles that provide broad representation of HLA class II that are prevalent in human populations [20]. Half maximal inhibitory concentrations (IC_{50}) were estimated and the predicted peptides were classified as exhibiting very high (<1 μM), high (1–10 μM), moderate (10–100 μM) or low (>100 μM) affinity. Peptides that exhibited very high, high or moderate affinity were considered binders (a more detailed classification is provided in Section 3).

The ability of predicted epitopes to bind HLA-A*0201 was assessed as previously described using a fluorescence polarization-based competitive peptide-binding assay [26]. The concentration of experimental peptide that inhibited 50% binding of the FITC-labeled reference peptide (IC_{50}) was determined. Experimental peptides were considered: high ($IC_{50} < 5$ μM), moderate ($5\ \mu M < IC_{50} < 50\ \mu M$) and low ($IC_{50} = 50–100$ μM) affinity binders. Peptides that failed to demonstrate dose-dependent inhibition or exhibited an $IC_{50} > 100$ μM were considered non-binders.

2.6. Human subjects

Whole-blood leukocyte reduction filters (blood filters; Sepacell RZ-2000, Baxter Healthcare Corporation, Irvine CA) were obtained from the Rhode Island Blood Center (Providence, RI). These used, de-identified filters contain white cells derived from blood donated with informed consent by healthy volunteers. The Lifespan Institutional Review Board (Rhode Island Hospital) approved this study.

2.7. Peripheral blood mononuclear cell (PBMC) recovery and purification

PBMCs were recovered from blood filters according to the methods of Meyer et al. [27]. Filters obtained within a 4-h period following the leukocyte depletion step were back-flushed at room temperature with Ca- and Mg-free Hank's basic salt solution containing sodium-EDTA and sucrose. The recovered leucocytes were purified by centrifugation on Ficoll-Paque Plus (1.077; Pharmacia, Uppsala, Sweden) gradient. All donors expressed HLA-A*0201 and HLA-DRB1.

2.8. Naïve PBMC cultures

The peptide-specific responses of naïve human T cells were induced by culturing fresh, purified PBMCs under conditions