

Dendritic cell-based vaccination against hepatitis C

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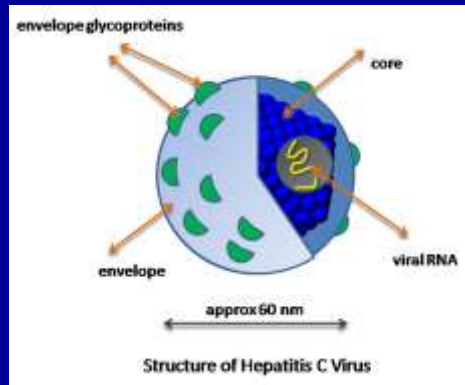
Hepatitis C: emerging infectious disease caused by hepatitis C virus (HCV)

- HCV major cause of liver disease (hepatitis, cirrhosis, carcinoma).
- Transmitted by contact infected blood/body fluids.
- High rate chronic infection, 170 million people world wide.
- No vaccine currently available.
- Outcome of standard treatment (ribavirin + pegylated interferon) is variable; often accompanied by significant side effects.
- New approaches (e.g., prophylactic or therapeutic vaccine) are need for prevention and treatment.



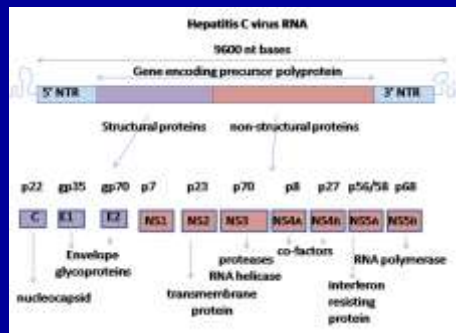
Hepatitis C virus

- Small, single-stranded RNA virus .
- RNA surrounded by a protective protein core.
- Lipid envelope of cellular origin.
- Two viral envelope glycoproteins (E1 and E2).



Viral genome

- Positive sense RNA genome consists of a single open reading frame of 9,600 nucleotide bases.
- Genome encodes core, envelope glycoproteins (E1, E2) and nonstructural (NS) proteins (NS1-NS5).
- Translated into single, 3,000 AA protein
- 3' and 5' ends are not translated; important in viral replication,
- cellular and viral proteases → 10 structural and non-structural proteins.



Genetically diverse: 6 major genotypes; >1M subtypes (quasispecies)

Distribution:

- 1a – common in North & South America; also found in Australia
- 1b – common in Europe and Asia
- 2a - most common genotype 2 in Japan and China
- 2b - most common genotype 2 in U.S. and Northern Europe
- 2c - most common genotype 2 in Western and Southern Europe
- 3a – most prevalent in Australia (40% of cases) and South Asia
- 4a – most prevalent in Egypt
- 4c - prevalent in Central Africa
- 5a - highly prevalent in South Africa only
- 6a - most common in S. China, Hong Kong, SE Asian countries

US: genotype 1 most common (~57% of patients infected with genotype 1a; ~17% infected with genotype 1b); ~10% and ~6% of patients infected with genotypes 2 and 3, respectively.



Genetic diversity

Cause: 1) rapid replication [10^{12} virions/day]; 2) RNA polymerase lacks fidelity and ability to correct errors
→ high rate mutation

Consequences:

1. Length and potential response to standard therapy (ribavirin + interferon) varies.
 - Genotypes 1 & 4 are less responsive; require 48 wks.
 - Genotypes 2 & 3 completed in 24 weeks
2. Infection with one genotype does not confer immunity against others; concurrent infections with two strains possible.
3. Virus readily evades immune response making treatment and vaccine development difficult.



Long-term goals: construct a therapeutic vaccine to treat chronic HCV infections; use dendritic cells as a delivery vehicle

Specific Aim I. Construct a DNA based vaccine

- A. Use computational and immunoinformatics tools to predict conserved, immunodominant human epitopes.
- B. Validate relevance of predicted epitopes by demonstrating the response of T cells obtained from patients exposed to HCV.
- C. Construct multi-epitope gene (vaccine) that encodes validated human epitopes.



Specific Aim II

- Transfect “humanized” (HLA-A*0201-/ HLA-DRB1*0101-transgenic H-2 class I/class II-knockout) mouse dendritic cells with vaccine construct.

Dendritic cells (DCs)

1. Antigen presenting cells.
 2. Play obligate role in initiating immune responses.
 3. Constitute rare, widely distributed cell population.
 4. FLT3 ligand: factor that stimulates DC production.
- Immunize humanized mice with vaccine-transfected DCs.
 - Quantify the resistance of immunized mice to challenge with recombinant vaccinia virus engineered to express HCV proteins.



Specific Aim III. Delineate immunity and the mechanisms that underlie the HCV-specific response in vaccinated mice

- *In vitro*: Quantify and compare HCV protein-specific cytolytic activity and cytokines produced by CD4⁺ and CD8⁺ T lymphocytes derived from vaccinated and control mice.
- *In vivo*: Pre-treat immunized mice with cytotoxic or neutralizing monoclonal antibody prior to challenge with recombinant HCV protein-expressing vaccinia virus to demonstrate the role of specific immune cell type or cytokine in resistance.



Specific Aim IA. Use computational and immunoinformatics tools to predict conserved, immunodominant HLA class I- and class II-restricted Hepatitis C virus epitopes (peptide)

- Identify conserved peptide sequences associated with genotype 1 and 5-10 additional peptides associated with genotypes 2 and 3 (Conservatrix).
- Determine potential ability of peptides to bind HLA molecules widely expressed by patient population (EpiMatrix).



HLA class I-restricted peptides (epitopes)

- Select 29 peptides (9-10 AA) associated with >90% genotypes 1A and 1B and varying degrees with genotypes 2 and 3.
- Among these, 7 peptides are highly conserved and associated with 90–100% of genotypes 2 and 3.
- These peptides predicted to bind HLA-A (A*0101, A*0201, A*0301, A*2402) or HLA-B (B*0702), alleles widely expressed by white, Afro-American and Hispanic populations.

Selected HLA class I-restricted peptides

LABEL	PEPTIDE	1A CVX	1B CVX	2A CVX	2B CVX	2C CVX	3A CVX	3B CVX	A0101	A0201	A0301	A2402	B0702
HCV-G1-CLASS1-01	TSCGNTLCY	99%	98%	--	4%	--	--	--	3.81		2.87		
HCV-G1-CLASS1-02	RVCEKMLLY	98%	100%	95%	100%	100%	--	--	2.61		2.73		
HCV-G1-CLASS1-03	LHGPTPLLY	98%	95%	--	4%	--	100%	--	2.75				
HCV-G1-CLASS1-04	KSTKVPAAV	98%	92%	--	100%	100%	100%	100%	2.57		1.91		
HCV-G1-CLASS1-06	WMNRLAFA	100%	99%	100%	100%	100%	88%	100%		2.48			
HCV-G1-CLASS1-07	MLVCGDDL	100%	--	100%	100%	100%	--	--		2.44			
HCV-G1-CLASS1-08	HMWNFISGI	98%	94%	90%	100%	100%	--	--		2.66		1.71	1.69
HCV-G1-CLASS1-09	SMDCNTCV	98%	99%	--	4%	--	--	--	1.95	2.6	1.91		
HCV-G1-CLASS1-10	NLGNIMYA	--	97%	--	4%	--	88%	--		2.8			
HCV-G1-CLASS1-11	AVCTRWAK	99%	99%	--	27%	--	100%	100%			2.52		
HCV-G1-CLASS1-12	DVCCSMYV	98%	94%	--	4%	--	--	--	2.52		2.42		
HCV-G1-CLASS1-14	VMGSSYGRV	98%	--	--	4%	--	--	--	2.09		2.44		
HCV-G1-CLASS1-16	TYSTYKFL	99%	99%	95%	54%	100%	100%	100%				3.48	
HCV-G1-CLASS1-18	NFISGIOYL	98%	95%	90%	100%	100%	--	--				2.43	
HCV-G1-CLASS1-19	FIWAKHMVNF	97%	97%	95%	96%	100%	--	--				3.11	
HCV-G1-CLASS1-20	PYRLWHYPC	88%	98%	95%	100%	100%	100%	100%				2.39	
HCV-G1-CLASS1-22	QPPTLLYRL	99%	96%	95%	100%	100%	100%	100%					2.97
HCV-G1-CLASS1-23	RPODVKFPG	97%	99%	85%	100%	100%	100%	--					2.35
HCV-G1-CLASS1-24	QPRRRRQPI	97%	98%	95%	100%	100%	75%	--					4.1
HCV-G1-CLASS1-25	QPGYWPPLY	94%	98%	--	--	--	100%	100%	1.86		2.28		2.54
HCV-CLASS1-02	ATDALMTGY	47%	34%	80%	88%	100%	13%	100%	4.09		2.8		
HCV-CLASS1-05	TGCFADLMGY	94%	96%	90%	100%	100%	100%	100%	2.78				
HCV-CLASS1-07	RLWHYPCV	35%	86%	100%	85%	100%	100%	100%		3.24			1.23
HCV-CLASS1-08	YLVAQATV	99%	86%	75%	12%	100%	--	100%		3.2			
HCV-CLASS1-10	ALSTGLHL	74%	97%	5%	--	--	100%	--		2.68		1.31	
HCV-CLASS1-12	HLFCHSKK	93%	88%	100%	85%	100%	100%	--			3.26		
HCV-CLASS1-13	KTKRNTNRR	91%	92%	85%	92%	100%	--	--			2.6		
HCV-CLASS1-17	OYLGLSLTL	100%	99%	90%	100%	100%	88%	100%				2.73	
HCV-CLASS1-22	LPCCSFIF	95%	95%	95%	100%	100%	100%	100%					3.48

HLA class II-restricted peptides (epitopes)

- Select 23 peptides (15-20 AA) associated with genotypes 1A and 1B.
- Four of these are highly conserved and also associated with genotypes 2 and 3.
- Select five additional peptides associated with genotype 2 and five with genotype 3.
- All are promiscuous epitopes predicted to bind multiple HLA class II (DRB1) alleles.



HLA class-II peptide binding

Peptide ID	Sequence	IC50 (nM)					
		Max Epitope Z-Score					
		'0101	'0201	'0401	'0701	'1101	'1501
HCV_G1_N62_734	Ac-IFPLLLLALPQRAYG-amide						
HCV_G1_DE0c_1946	Ac-AGDQYVWNPVAVATLDFG-amide						
HCV_G1_N64c_1870	Ac-VGLLVNLAALSPGQ-amide						
HCV_G1_2879	Ac-LDNDRHLGSLFSLFYS-amide						
HCV_G1_N64c_1769	Ac-SGDTLAGLSTLPMPA-amide						
HCV_G1_1941	Ac-AARYDGLSLTQLKRLHGM-amide						
HCV_G1_2440	Ac-KLPNALHGLRPH-amide						
HCV_G1_N64c_1725	Ac-AEFGKGLGGLGTAARGAE-amide						
HCV_G1_2445	Ac-LDLSKERVKAASVYVAVL-amide						
HCV_G1_2840	Ac-IRVARELMTFFYSVLARDQLED-amide						
HCV_G1_N64c_1750	Ac-LMMAFSAVTSPLTTS-amide						
HCV_G1_N64c_1758	Ac-TSPLTSDGLFNLGGDY-amide						
HCV_G1_N62_752	Ac-VTGVNMLDGLDEALELT-amide						
HCV_G1_ENV_265	Ac-AALRHDLVDSATLCSALY-amide						
HCV_G1_1035	Ac-DMRWVGLSLKPTLADPTP-amide						
HCV_G1_2841	Ac-GRTEFMAVWRKVAL-amide						
HCV_G1_N62_800	Ac-VPTFVIVGGLNLCAARAAV-amide						
HCV_G1_N64c_1810	Ac-EGAVQMNMLAFASRQ-amide						
HCV_G1_N64c_1888	Ac-LTPTKTIQDGLPALPQY-amide						
HCV_G1_2013	Ac-VPLRVVWRHARSVRAKLLSQQGR-amide						
HCV_G1_N62_748	Ac-LENEVLNAEAVGAWH-amide						

Peptide ID	Sequence	IC50 (nM)					
		Max Epitope Z-Score					
		'0101	'0201	'0401	'0701	'1101	'1501
HCV_G1_N64c_1759	Ac-VKGMDFSAHLSPLSTG-amide						
HCV_G1_752	Ac-LEKLVVHRAASAEINGL-amide						
HCV_G1_ENV1_715	Ac-KYVYVWVWVYVLLFLLADRVGAC-amide						
HCV_G1_N64c_1907	Ac-GLTSLRLLRNWITSCS-amide						
HCV_G1_2863	Ac-VRMWRHFFSLAGDTLQNLN-amide						
HCV_G1_N62_807	Ac-YPRLDFPKLVAVGDTPLVIG-amide						
HCV_G1_2483	Ac-IRVALLVYTSGRASASQRF-amide						
HCV_G1_Phdap/NSP_201	Ac-VERLLKQWTSKXPLQLLQ-amide						
HCV_G1_N64c_1594	Ac-VEDPKVLSAKMPALPG-amide						
HCV_G1_N61_409	Ac-SGNGLVINGGWNSTALNND-amide						

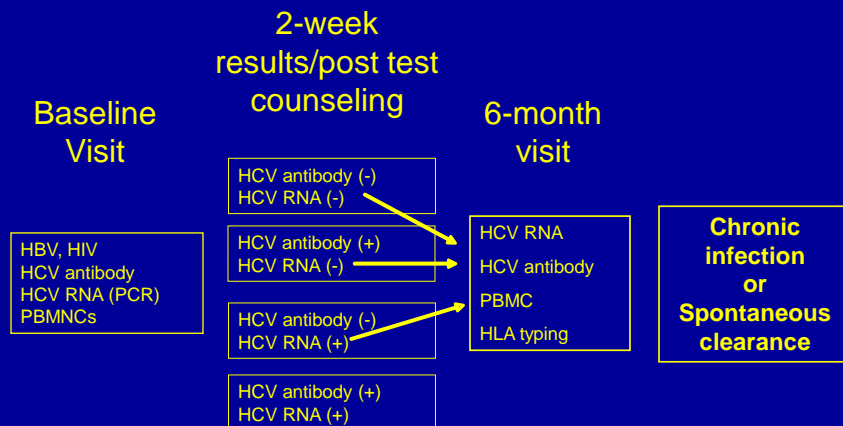
IC50 (nM)
IC50 (nM)
IC50 (nM)



Specific Aim IB. Validate relevance of predicted epitopes by demonstrating the response of T cells obtained from patients exposed to HCV.



Schema: Clinical determination HCV status high risk young adults



Initial Screening 18-25 year olds Adult Corrections Institution, RI¹

% HCV infected	10% (1/10) ^{2,3}
% IVDU	60%
Mean age 1st IV drug use	17 yrs (14 – 24yrs)
% Share needles (unsure or known HCV ⁺ individual)	50%
1 st ever HCV screen	100%

¹ 14 young men approached; 10 consented to screening.

² Prior studies 30% prevalence of HCV in ACI

³ Genotype 3a

Correlates of an Effective Immune response to HCV

PBMCs derived from: chronic HCV-infected;
spontaneous clearers; exposed but not infected

- Flow cytometric analysis of T cell populations and subpopulations:
 - State of differentiation (CD28, CD57)
 - Inhibitory receptors: CD279 (PD-1), CD272 (BTLA), CD152 (CTLA-4), Tim3
 - T regulatory cells (CD25, CD127)
- Response to predicted, HCV epitopes
 - Proliferation
 - EliSpot assay
 - Cytokine bead array analysis

Specific Aim 1C. Construct multi-epitope gene (vaccine) that encodes validated human epitopes

- DNA sequences that encode validated peptides (epitopes) that elicit greater responses by clearers vs. non-clearers will be incorporated into “string of beads” multi-epitope HLA class I and HLA class II vaccine constructs:



Specific Aims 2 and 3. Immunize humanized HLA-A*0201-/ HLA-DRB1*0101-transgenic mice with vaccine-transfected DCs and assess immune response.

