

## PRODUCTION OF RABBIT SPECIFIC ANTIBODIES AGAINST CONSERVED AND POTENTIALLY IMMUNOGENIC PEPTIDES OF THE HCV ENVELOPE GLYCOPROTEIN E2

Simões, R.S.Q<sup>1,2,3\*</sup>; Rodríguez-Lázaro. D<sup>2,3</sup>

<sup>1\*</sup> Oswaldo Cruz Foundation, Fiocruz, Avenida Brasil, 4.365, Mangueiras, Rio de Janeiro 21040-900, Brazil. E-mail: [rachelsqsimoes@gmail.com](mailto:rachelsqsimoes@gmail.com)

<sup>2</sup> Microbiology Division, Faculty of Sciences, University of Burgos, 09001 Burgos, Spain.

<sup>3</sup> Research Centre for Emerging pathogens and Global Health, University of Burgos, 09001 Burgos, Spain

### INTRODUCTION

Hepatitis C virus (HCV) is a chronic infection that cause of hepatocellular carcinoma, and end-stage liver diseases. It is a positive-sense RNA virus of the *Flaviviridae* family that can be grouped into six genotypes differed by approximately 30% of the nucleotide sequence. Envelope (E1 /E2) proteins of HCV may generate neutralizing antibodies. At the end N-terminus of the E2 protein is a region characterized by a high degree of variability called hypervariable region 1 (HVR1), which corresponds to the 27 amino acids of the E2 protein, very important in neutralizing HCV. Despite the high variability of E2 protein some amino acid position are highly conserved. This study was designed to assess the immunogenic properties of conserved peptides derived from E2 HVR1 regions. Nucleotide sequences of all available HCV E2 region in the databases were aligned and four amino acid sequences with immunogenic potential were selected.

### MATERIAL AND METHODS

We selected four neutralizing peptides from the sequence AJ238799 Japanese HVRI in different positions of aminoacids: peptide 1 (aa412-149), peptide 2 (517-531), peptide 3 (aa 523-535) and peptide 4 (aa 373-416). These peptides were synthesized conjugated with KLH and resuspended in PBS (2µg/µL). Each peptide (0.5 mg/mL) was used to inoculate one rabbit after emulsification of volume of Freund's complete adjuvant. These synthetic peptides located downstream of the hypervariable region I within the HCV E2 protein were inoculated into the five rabbits (one peptide for each animal). Each peptide was injected as conjugated to KLH and one animal was injected with 0,5 ml saline solution at the 14 days intervals of immunization protocol to serve as controls. Each rabbit was immunized with a dose containing 0.5 mg/mL. Equal volumes of diluted KLH – peptide and Freund's complete adjuvant were emulsified and injected subcutaneously into the rabbit in three different sites. Before each immunization was collected pre-immune serum through the auricular vein of each rabbit and new serum samples immunes carried out post-immunization. On day 0 each rabbit was immunized again with the same protein emulsified with Complete Freund's adjuvant and day 14 and day 28, each rabbit was immunized again with the same protein emulsified with Incomplete Freund's adjuvant. One month after the last immunization, the animals were anesthetized with ketamine and xylazine to bleeding of the rabbits by cardiac puncture.

Table 1: Synthetic peptides with immunogenic potential

Peptide (3mg of each)	Sequence	Concentration after resuspension in PBS
412-419	QLVNTNGS	2 µg/µL
517-531	GTTDHVGVPTDYDWGK	2 µg/µL
523-535	GAPTYSWGANDTD	2 µg/µL
373-416	VKLLFAGVDGGTYVTGGTMAKNTLGLTSLFSPGSSQKQLVNT	1,6 µg/µL

Table 2: Inoculation of each peptide per animal by subcutaneous injections

Animal	Peptide	Dose	Volume	Via
1	412-419 KLH	0,5 mg/dose/animal	250 µL de peptídeo + 250 µL de adjuvante = 0,5mL	SC
2	517-531 KLH	0,5 mg/dose/animal	250 µL de peptídeo + 250 µL de adjuvante = 0,5mL	SC
3	523-535 KLH	0,5 mg/dose/animal	250 µL de peptídeo + 250 µL de adjuvante = 0,5mL	SC
4	HVR1	0,5 mg/dose/animal	250 µL de peptídeo + 250 µL de adjuvante = 0,5mL	SC
5	Control	0,5 mg/dose/animal	250 µL de peptídeo + 250 µL de adjuvante = 0,5mL	SC

### RESULTS AND CONCLUSIONS

The serums from rabbits immunized with peptide were tested to quantify the titer of relevant immunoglobulin using protocol ELISA in house sensitizing the plates with peptide conjugated to BSA. All peptides shown to be highly immunogenic and neutralizing with detectable levels of specific antibodies appeared at the first determination 15 days post immunization increasing after 30 days showing better response to the peptide 4(aa 373-416). The high concentrations of serum showed high level of cross-reactivity with exception of anti-HVR1 antibody which shows beyond the specific reaction, a nonspecific reaction with BSA. In conclusion, all the designed peptides were able to generate anti-E2 specific antibodies in rabbits at relatively high titles.

### RESULTS AND CONCLUSIONS

Table 3: Possible cross-reactivity between the peptides and pre-immune serum of the five rabbits that were further immunized were tested by in house Elisa

Antibodies (Serum)	Antigens (Peptides)			
	412-419 BSA	517-531 BSA	523-535 BSA	HVR1 BSA (373-416)
Serum Rabbit sacrificed PBS 1/300	0.04	0.09	0.08	0.55
Serum Rabbit sacrificed PBS 1/600	0.04	0.05	0.02	0.31
Serum Rabbit Pre-Immune 412-419 1/300	0.04	0.09	>3	0.07
Serum Rabbit Pre-Immune 412-419 1/600	0.04	0.06	2.71	0.05
Serum Rabbit Pre-Immune 523-535 1/300	0.02	0.07	0.04	0.03
Serum Rabbit Pre-Immune 523-535 1/600	0.03	NR	0.02	NR
Serum Rabbit Pre-Immune HVR1 1/300	0.03	0.06	0.16	0.04
Serum Rabbit Pre-Immune HVR1 1/600	0.02	NR	0.06	NR

Table 4: Standardization of dilutions of immune sera anti-peptide in the in house Elisa

Antibodies (Serum)	Antigens (Peptides)			
	HVR1 BSA (373-416)	412-419 BSA	517-531 BSA	523-535 BSA
Serum Rabbit sacrificed 517-531 1/300	>3	1,51	>3	1,00
Serum Rabbit sacrificed 517-531 1/600	2,25	1,44	>3	0,45
Serum Rabbit Pre-Immune 517-531 1/1.200	1,39	0,99	>3	0,24
Serum Rabbit Pre-Immune 517-531 1/2.4000	0,90	0,78	>3	0,09
Serum Rabbit Pre-Immune 517-531 1/4.800	0,59	0,63	>3	0,14
Serum Rabbit sacrificed 523-535 1/600	2,23	0,44	>3	>3
Serum Rabbit sacrificed 523-535 1/1.200	1,56	0,37	>3	>3
Serum Rabbit Pre-Immune 523-535 1/2.400	1,11	0,17	>3	>3
Serum Rabbit Pre-Immune 523-535 1/4.800	0,66	0,09	2,93	>3
Serum Rabbit sacrificed HVR1 1/300	>3	>3	>3	>3
Serum Rabbit sacrificed HVR1 1/600	>3	>3	>3	>3
Serum Rabbit Pre-Immune HVR1 1/1.200	>3	>3	>3	>3
Serum Rabbit Pre-Immune HVR1 1/2.4000	>3	>3	>3	>3
Serum Rabbit Pre-Immune HVR1 1/4.800	>3	>3	>3	>3
Serum Rabbit sacrificed 412-419 1/300	2,72	>3	0,08	0,83
Serum Rabbit sacrificed 412-419 1/600	1,78	>3	0,04	0,39
Serum Rabbit Pre-Immune 412-419 1/1.200	0,84	2,02	0,02	0,14
Serum Rabbit Pre-Immune 412-419 1/2.400	0,43	1,27	0,01	0,06
Serum Rabbit Pre-Immune 412-419 1/4.00	0,22	0,75	0,01	0,25

Table 5: Appropriate dilutions for each antibody that allows specific detection of anti-E2 antibodies generated in rabbits

Antibodies (Serum)	Antigens (Peptides)				
	HVR1 BSA	412-419 BSA	517-531 BSA	523-535 BSA	BSA
Serum Rabbit sacrificed HVR1 1/7.000	1.64	1.60	1.62	1.45	1.70
Serum Rabbit sacrificed HVR1 1/7.000	1.83	1.82	1.71	1.56	1.94
Serum Rabbit sacrificed 412-419 1/1.500	0.24	0.94	0.01	0.05	0.01
Serum Rabbit sacrificed 412-419 1/1.500	0.08	1.24	0.01	0.02	0.01
Serum Rabbit sacrificed 517-531 1/4.800	0.12	0.12	0.75	0.03	0.01
Serum Rabbit sacrificed 517-531 1/4.800	0.14	0.16	0.86	0.05	0.01
Serum Rabbit sacrificed 523-535 1/7.000	0.12	0.05	0.41	0.82	0.05
Serum Rabbit sacrificed 523-535 1/7.000	0.12	0.05	0.38	0.86	0.02

Table 6: HVR1 antiserum reacts strongly with BSA, but if it plate is covered with peptides conjugated with KLH, the reaction is specific

Antibodies (Serum)	412-419 BSA	412-419 KLH	517-531 BSA	517-531 KLH	523-535 BSA	523-535 KLH	HVR1 BSA	HVR1 KLH	HVR1 PURO	BSA
Serum Rabbit sacrificed HVR 1/7.000	1.60	0.25	1.62	0.52	1.45	0.19	1.64	1.86	1.10	1.70
Serum Rabbit sacrificed HVR 1/7.000	1.82	0.23	1.71	0.56	1.56	0.22	1.83	1.88	1.14	1.94

### FINANCIAL SUPPORT