

2014

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Mishra, S., Losikoff, P. T., Self, A. A., Terry, F., Ardito, M. T., Tassone, R., . . . Gregory, S. H. (2014). Peptide-pulsed dendritic cells induce the hepatitis C viral epitope-specific responses of naïve human T cells. *Vaccine*. <http://dx.doi.org/10.1016/j.vaccine.2014.03.083>

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3 **Peptide-pulsed Dendritic Cells Induce the Hepatitis C Viral**
4 **Epitope-specific Responses of Naïve Human T Cells**
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15 **Abbreviations:** DCs, dendritic cells; HCV, hepatitis C virus; ICS, immunogenic consensus
16 sequences; NS, nonstructural; PBMC, peripheral blood mononuclear cell; SVR, sustained virologic
17 response
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25 **Abstract**

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

44

45 **Keywords.** dendritic cell; EpiMatrix; epitope; HCV; vaccine; T cell

46

46 **Introduction**

47 Hepatitis C virus (HCV), a small single-stranded RNA virus, constitutes a major cause of liver
48 disease [1]. The positive-sense genome encodes an ~3,000 amino acid poly-protein precursor,
49 which is cleaved by cellular and viral proteases to yield three structural [core, envelope 1 (E1) and
50 E2], and seven nonstructural (p7, NS2, NS3, NS4a, NS4b, NS5a and NS5b) proteins [2].
51 Spontaneous resolution of HCV infections is associated with broad, MHC class I- (CD8⁺) and class
52 II-restricted (CD4⁺) T cell responses to multiple viral epitopes derived from these proteins [3,4].
53 Unfortunately, only 20% of patients clear infection spontaneously, most develop chronic disease [5].
54 Seventy to eighty percent of patients infected with HCV genotype 1 (the principal causative agent of
55 hepatitis C in the U.S.) experience a sustained virologic response (SVR) following treatment that
56 includes protease inhibitors, i.e., telaprevir or boceprevir, administered in conjunction with
57 PEGylated interferon and ribavirin. A significant number of those treated remains infected, however,
58 the cost of treatment is high, and the risk and severity of side effects are considerable [6,7]. New
59 approaches to treating chronic HCV infections are urgently needed.

60 Therapeutic vaccination concurrent with or without drug therapy offers an additional approach
61 to treating chronic hepatitis C. Indeed, the capacity of a significant percentage of patients to resolve
62 acute infections spontaneously suggests that an effective therapeutic vaccine is a realistic goal. A
63 safe and effective vaccine must elicit broad, vigorous CD4⁺ and CD8⁺ T cells responses to
64 conserved viral epitopes, which culminate in the elimination of HCV without causing liver pathology.
65 Development of such a vaccine has proven problematic, however, due primarily to: infidelity of the
66 viral RNA polymerase (NS5b), genetic diversity and the rapid emergence of viral variants [8]. To
67 date, a number of vaccine strategies have demonstrated negligible or only limited success in clinical
68 trials [9,10].

69 Vaccination with HCV epitope expressing dendritic cells (DCs) offers a vector-mediated
70 approach to treating chronic, HCV infected patients. DCs play a central role in CD4⁺ and CD8⁺ T cell

71 activation and the induction of immunity [11]. The potential effectiveness of DC-based vaccines in
72 treating chronic hepatitis C has been demonstrated in animal models [12-14]. Moreover, in a recent
73 Phase I clinical trial, chronically-infected patients vaccinated with monocyte-derived DCs pulsed with
74 6 HCV-specific, HLA-class I-restricted peptides exhibited peptide-specific CD8 T cell responses [15].
75 These responses were not sustained, however, and there was no effect on viral load suggesting that
76 HCV clearance might require vaccination with DCs that expressed a broader range of viral epitopes.
77 Toward this end, immunoinformatics tools were used to predict 21 HLA-A*0201-restricted epitopes
78 and 19 HLA-DRB1-restricted immunogenic consensus sequences (ICS, each composed of multiple
79 epitopes), which were highly-conserved and encoded by HCV genotype 1. These predicted
80 epitopes/ICS were synthesized as peptides and their capacities to bind HLA molecules were
81 determined. Subsequently, their immunogenicity and ability to elicit the peptide-specific responses of
82 naïve human T cells were validated in an *in vitro* peripheral blood mononuclear cell (PBMC)
83 immunogenicity assay. Similarly, monocyte-derived DCs pulsed with these same peptides induced
84 the epitope-specific responses of naïve CD4⁺ and CD8⁺ T cells in culture demonstrating the potential
85 efficacy of a multi-epitope-based HCV vaccine that targets dendritic cells.

86

87

88

88 **Materials and Methods**

89 **Genome Collection**

90 Hepatitis C viral sequences marked complete, representing a total of 871 genotype 1a and genotype
91 1b polyproteins, were acquired from the Los Alamos sequence and immunology database [16,17].

92 **Conserved 9-mer search**

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

98 **Epitope mapping**

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

107 **Immunogenic consensus sequences**

108 HLA-DRB1-restricted ICS were constructed using EpiAssembler, an algorithm that maximizes
109 epitope density by assembling potentially immunogenic 9-mers (identified using EpiMatrix) into 18-
110 25 amino acid stretches [23]. To avoid potential cross-reactivity with the human proteome, any
111 peptide that shared more than 7 identities per 9-mer frame was eliminated from further consideration

112 [24]. The final HLA-A2- and –DRB1-restricted peptide sequences were synthesized using 9-
113 fluoronylmethoxycarbonyl chemistry and purified >85% by 21st Century Biochemicals
114 (Marlboro, MA).

115 **HLA Binding Assay**

116 The capacity of predicted epitopes (peptides) to bind multiple HLA-DRB1 alleles was assessed
117 using a competitive, HLA class II binding assay as we described previously [24,25], using HLA
118 molecules obtained from Bill Kwok, Benaroya Research Institute, Seattle, WA). Assays were
119 performed for HLA-DRB1*0101, -DRB1*0301, -DRB1*0401, -DRB1*0701, -DRB1*1101 and -
120 DRB1*1501, alleles that provide broad representation of HLA class II that are prevalent in human
121 populations [20]. IC₅₀ values were estimated and the predicted peptides were classified as exhibiting
122 very high (< 1 μM), high (1 μM - 10 μM), moderate (10 μM - 100 μM) or low (>100 μM) affinity.
123 Peptides that exhibited very high, high or moderate affinity were considered binders (a more detailed
124 classification is provided in the results section).

125 The ability of predicted epitopes to bind HLA-A*0201 was assessed using a fluorescence
126 polarization-based competitive peptide-binding assay according to published methods [26]. The
127 concentration of experimental peptide that inhibited 50% binding of the FITC-labeled reference
128 peptide (IC₅₀) was determined. Experimental peptides were considered: high (IC₅₀<5 μM), moderate
129 (5 μM<IC₅₀<50 μM) and low (IC₅₀ = 50 - 100 μM) affinity binders. Peptides that failed to demonstrate
130 dose-dependent inhibition or exhibited an IC₅₀>100 μM were considered non-binders.

131 **Human subjects**

132 Whole-blood leukocyte reduction filters (blood filters; Sepacell RZ-2000, Baxter Healthcare
133 Corporation, Irvine CA) were obtained from the Rhode Island Blood Center (Providence, RI). These
134 used, de-identified filters contain white cells derived from blood donated with informed consent by

135 healthy volunteers. The Lifespan Institutional Review Board (Rhode Island Hospital) approved this
136 study.

137 **Peripheral blood mononuclear cell (PBMC) recovery and purification**

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

143 **Naïve PBMC cultures**

144 The peptide-specific responses of naïve human T cells were induced by culturing purified PBMCs
145 under conditions described by others and modified in our laboratory [28]. PBMCs, $2.5 \times 10^5/200 \mu\text{l}$
146 serum-free X-VIVO 15 medium (Lonza, Walkerville, MD) supplemented with glutamine, penicillin,
147 streptomycin, and 50 U/ml recombinant human IL-2 (Roche Applied Science, Indianapolis, IN), were
148 transferred to 96-well round bottom tissue culture plates and incubated 14 days with single (10
149 $\mu\text{g/ml}$) HLA-A2- or -DRB1-restricted peptide. Half the spent medium was replaced with fresh medium
150 containing IL-2, but no additional peptide, on days 3, 7 and 10 of the incubation period.

151 **ELISpot assays**

152 Cells collected after 14 days incubation under the culture conditions described were washed and
153 resuspended in X-VIVO 15 medium supplemented with glutamine and antibiotics. The frequency of
154 epitope-specific T lymphocytes was quantified using human IFN- γ ELISpot assay kits purchased
155 from eBioscience, Inc. (San Diego, CA) and the protocol provided. Triplicate wells were inoculated
156 with 50,000 cells/200 μl X-VIVO 15 medium and 10 $\mu\text{g/ml}$ of the same peptide present during the
157 pre-stimulation period. Positive (phytohemagglutinin) and negative (0.1% DMSO) controls were
158 included.

159

159 **DC priming of naïve T cell *ex vivo***

160 Monocyte-derived DCs were generated *in vitro* in accordance with methods described by others
161 [29]. CD14⁺ monocytes were purified from PBMCs back-flushed from blood filters using anti-CD14-
162 coated magnetic beads (Miltenyi Biotec; Auburn, CA). T lymphocytes were purified by negative
163 selection using the human pan T cell isolation kit II also purchased from Miltenyi. Biotin-conjugated
164 anti-mouse CD25 (Miltenyi) was added to the biotin-conjugated antibody cocktail supplied with the
165 kit to eliminate CD25⁺ regulatory T cells. Purified T lymphocytes were frozen and stored in liquid
166 nitrogen for later use. The purified CD14⁺ monocytes were suspended in X-VIVO 15 medium that
167 contained L-glutamine, penicillin, streptomycin, 100 ng/ml GM-CSF (PreproTech, Rocky Hill, NJ)
168 and 25 ng/ml IL-4 (PeproTech), and cultured in 24-well, ultra-low attachment tissue culture plates
169 (Corning Inc., Lowell, MA). Half the medium was replenished with fresh medium containing GM-CSF
170 and IL-4 on day 3. The resultant DC population was collected on day 5; the cells were suspended in
171 fresh X-VIVO medium, transferred to individual wells of a 96-well, round bottom plate (1×10^4
172 cells/200 μ l medium) and pulsed with single (10 μ g/ml) peptides. On the following day, 25 ng/ml
173 TNF- α (PeproTech) was added to promote DC maturation. After another 48 hours incubation, 100 μ l
174 of medium was removed, 6×10^5 naïve T lymphocytes (derived from the population purified and
175 frozen above)/100 μ l X-VIVO medium were added to each well; and the cells were co-cultured.

176 To quantify the epitope-specific responses of purified T cells co-cultured with peptide-pulsed
177 DCs, the cells were collected after 14 days incubation, centrifuged and resuspended in fresh X-VIVO
178 15 medium. The cells (50,000/100 μ l) were transferred to IFN- γ capture antibody-coated ELISpot
179 plates that contained 5,000 mature DCs/100 μ l X-VIVO 15/well generated as described in the
180 previous section and pulsed with the same peptide present during the initial co-culture period. The
181 remainder of the assay was conducted according to the supplier's protocol referenced in the
182 preceding section.

183

184 **Results**

185 **Epitope/ICS predictions**

186 A set of 21, HLA-A*0201-restricted epitopes were selected for synthesis from a set of more than 100
187 highly conserved, high-scoring peptides predicted using the EpiMatrix and Conservatrix algorithms
188 (Table I). Twenty of these epitopes scored in the top 1% (Z score ≥ 2.32) and one (peptide ID# 13)
189 scored in the top 5% (Z score ≥ 1.64) of predicted binders, indicating a very high probability of HLA-
190 A*0201 binding. The amino acid sequence, A*0201 EpiMatrix Z Scores and relative conservation of
191 these peptides among HCV genotypes 1a and 1b isolates (calculated using Conservatrix) are shown
192 in Table II.

193 Twenty-five HLA-DRB1-restricted ICS from the input HCV genotype 1a and 1b sequences
194 were selected from a list of 79 that were predicted using the EpiAssembler algorithm; these varied
195 from 15 to 27 amino acids in length. Each was composed of multiple, overlapping 9-mers (epitopes)
196 capable of binding more than one HLA-DRB1 allele. The number of epitopes contained within each
197 ICS predicted to bind across all 8 alleles was determined and ranged from 5 to 28 epitopes/ICS.
198 Among the original 25 ICS, three exhibited significant homology to sequences found in the human
199 genome and three overlapped protein junctions located within the HCV poly-protein; these six ICS
200 were excluded from further study. The amino acid sequence, HLA-DRB1 EpiMatrix Z scores,
201 number of predicted epitopes and conservation of the 19 remaining ICS among HCV genotypes 1a
202 and 1b isolates are shown in Table II.

203 **Binding analyses**

204 Each predicted epitope/ICS was synthesized. HLA binding affinity and concurrence with
205 computational predictions were determined. Positive predictions were defined as epitope scores
206 ≥ 1.64 on the EpiMatrix Z-scale and HLA binding of $IC_{50} < 100 \mu M$. Overall, the proportion of
207 predictions that concurred with binding was $\sim 90\%$ (19/21) and 84% (96/114) for the HLA-A*0201-

208 restricted peptides (Table III) and HLA–DRB1-restricted ICS (Table IV), respectively. We expect no
209 more than three to five percent of randomly selected peptides to bind to any given HLA. In the
210 current case, approximately 85% of all predictions were confirmed, consistent with our previous
211 experience. The small number of negative predictions present in this dataset makes statistical
212 validation of the correlation between predicted binding and observed results difficult. We suggest
213 that the correlation between prediction and observed outcome is self-evident.

214 **Epitope/ICS validation.**

215 All predicted A2-restricted epitopes and DRB1-restricted ICS that exhibited HLA binding affinity
216 induced naïve T cell reactivity and IFN- γ production *ex vivo*, validating their immunogenicity.
217 PBMCs obtained from healthy blood donors and cultured 14 days in the presence of single peptides
218 subsequently exhibited peptide-specific IFN- γ production in ELISpot assays (Figure 1). Similar
219 results were obtained when purified, naïve human T lymphocytes were co-cultured with CD14⁺
220 monocyte-derived DCs pulsed with peptides. DCs pulsed with single HLA-A2-restricted or –DRB1-
221 restricted peptides induced marked increases in IFN- γ production by naïve human T cells in ELISpot
222 assays (Figure 2). Notably, all peptide sequences induced IFN- γ production, but not to the same
223 extent in co-cultures derived from different blood donors.

224

225

226

226 **Discussion**

227 Triple drug therapy (telaprevir or boceprevir, combined with PEGylated interferon and ribavirin) is
228 recommended by the American Association for the Study of Liver Diseases as the standard
229 treatment for unresolved HCV genotype 1 infections [30]. The SVR rate is improved significantly in
230 patients who receive triple therapy compared to those treated with PEGylated interferon and ribavirin
231 alone. While the outcome is improved, the overall success of triple drug therapy is limited by a
232 variety of factors that include: general access to health care, cost of therapy, patient compliance,
233 drug-drug interactions, emergence of anti-viral resistant variants, adverse side effects, and host
234 factors that affect relative effectiveness [30,31]. Indeed, logistics and expense are major deterrents
235 in treating chronically infected patients with antiviral drugs worldwide. As such, the development of a
236 safe, effective and affordable vaccine represents the best hope for bringing the global hepatitis C
237 epidemic under control, a stated objective in the US Department of Health and Human Services'
238 Viral Hepatitis Action plan [32].

239 A safe and effective therapeutic vaccine for chronic hepatitis C must elicit broad, vigorous
240 CD4⁺ and CD8⁺ T cell responses to conserved viral epitopes, which result in viral elimination in the
241 absence of significant liver injury. To date, four distinct vaccine strategies have demonstrated only
242 limited success in clinical trials: recombinant protein, peptide, genetic or DNA-based and vector-
243 mediated [9,10,33]. Recombinant protein vaccines are safe and well tolerated, but generally
244 ineffective owing to their inability to induce a CD8⁺ T cells. Peptide-based HCV vaccines are similarly
245 ineffective despite their ability to elicit weak, epitope-specific T cell responses; only a transient
246 reduction in viral load was determined in a minority of chronically-infected patients [34,35]. Similarly,
247 patients vaccinated with a codon-optimized HCV NS3/4A DNA construct exhibited only a transient
248 reduction in serum viral load [36]. Finally, just half of the chronically-infected patients vaccinated in a
249 Phase I clinical trial with modified vaccinia virus Ankara [MVA] engineered to express HCV NS3-5B
250 proteins exhibited a temporary reduction in viral load [37].

251 Therapeutic vaccination with HCV epitope-expressing DCs concurrent with or without
252 chemotherapy offers an additional vector-mediated approach to treating chronic, HCV-infected
253 patients. The utility of this approach is supported by clinical trials demonstrating tumor regression
254 and long-term survival in a subset of cancer patients administered antigen-pulsed DCs [38]. Indeed,
255 FDA approval of Dendreon's prostate cancer vaccine, Sipuleucel-T (Provenge), demonstrates the
256 feasibility of developing a DC-vectored therapeutic vaccine for chronic hepatitis. The potential
257 efficacy of DC-based vaccines in treating chronic hepatitis C was demonstrated in a recent Phase I
258 clinical trial in which patients vaccinated with monocyte-derived DCs pulsed with 6 HCV-specific,
259 HLA-class I-restricted peptides exhibited peptide-specific CD8 T cell responses [15]. These
260 responses were not sustained, however, nor was an effect on viral load discerned. The failed
261 response of patients vaccinated with peptide-pulsed DCs in this clinical trial was attributed to the
262 dearth of viral epitopes presented [15]. Immunization with DCs that express a broad array of HLA
263 class I- and class II-restricted viral epitopes offers a means of significantly enhancing vaccine
264 efficacy.

265 The data presented herein support the EpiMatrix algorithm as an approach to high-volume
266 genomic screening for vaccine candidates. Nineteen of 21 predicted, HLA class I-restricted peptides
267 (>90%) were bound by HLA A*0201 *in vitro*, in accordance with previously published data [39]. All
268 predicted HLA-class II-restricted peptides were bound by at least three HLA-DRB1 alleles,
269 substantiating their promiscuity and potential recognition by a large, diverse human population [20].
270 A large, retrospective comparison conducted previously found EpiMatrix was >75% accurate across
271 the HLA-DRB1 alleles studied here, and more accurate than all other epitope mapping algorithms in
272 the public domain [22]. In the present study, 96 of the 114 HLA-DRB1-peptide pairs exhibited
273 binding as predicted. Conceivably, a number of factors (e.g., peptide folding or aggregation under
274 assay conditions, and the accuracy of immunoinformatic algorithms) contribute to the lack of a strict
275 correlation between predicted and actual binding.

276

277 **Conclusion**

278 IFN- γ is a principal mediator of anti-HCV-specific T cell responses [40,41]. All the predicted, HLA-
279 A2-restricted and –DRB1-restricted peptides that exhibited HLA binding activity also induced IFN- γ
280 production in cultures of naïve human PBMCs, thus validating their immunogenicity and the
281 prognostic accuracy of the EpiMatrix algorithm. Similarly, monocyte-derived DCs pulsed with the
282 same peptides and co-cultured with naïve CD4⁺ and CD8⁺ human T cells induced epitope-specific
283 IFN- γ production. This latter observation supports the therapeutic potential of DCs pulsed *ex vivo*
284 with a broad array of HLA-A2- and –DRB1-restricted epitopes in treating chronic, HCV-infected
285 patients. This approach suffers, however, from a number of practical limitations, foremost of which is
286 the estimated high cost of treatment. Instead, we are currently exploring alternative strategies that
287 include delivering the epitopes validated herein to DCs *in situ*. Regardless, epitope-driven
288 immunotherapy alone or in combination with chemotherapy offers an additional means of treating
289 the expanding patient population affected by chronic hepatitis C.

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292

292 **Acknowledgements**

293 Supported by National Institutes of Health Research Grant U19 AI082642.

294

295 **Authors' contributions**

296 SM, PTL and SHG: experimental design and performance, data analyses, manuscript preparation;

297 AS and RT: experimental performance and data acquisition; FT, MA, WM and ADG: EpiMatrix

298 analysis, interpretation and discussion. All authors have read and approve of this article.

299

300 **Disclosures**

301 Anne De Groot and William Martin are senior officers and majority shareholders at EpiVax, Inc.

302 These authors acknowledge a potential conflict of interest and attest that the work contained in this

303 report is free of any bias that might be associated with the commercial goals of the company. None

304 of the remaining co-authors has any potential financial conflict of interest related to the manuscript to

305 disclose.

306

306 **References**

- 307
308 [1] D. Moradpour, F. Penin, C.M. Rice, Replication of hepatitis C virus, *Nat. Rev. Microbiol.* 5
309 (2007) 453-463 .
- 310 [2] A. Grakoui, C. Wychowski, C. Lin, S.M. Feinstone, C.M. Rice, Expression and identification of
311 hepatitis C virus polyprotein cleavage products, *J. Virol.* 67 (1993) 1385-1395 .
- 312 [3] G. Missale, R. Bertoni, V. Lamonaca, A. Valli, M. Massari, C. Mori, M.G. Rumi, M. Houghton,
313 F. Fiaccadori, C. Ferrari, Different clinical behaviors of acute hepatitis C virus infection are
314 associated with different vigor of the anti-viral cell-mediated immune response, *J. Clin. Invest*
315 98 (1996) 706-714 .
- 316 [4] F. Lechner, D.K. Wong, P.R. Dunbar, R. Chapman, R.T. Chung, P. Dohrenwend, G. Robbins,
317 R. Phillips, P. Klenerman, B.D. Walker, Analysis of successful immune responses in persons
318 infected with hepatitis C virus, *J. Exp. Med.* 191 (2000) 1499-1512 .
- 319 [5] H.B. El Serag, Hepatocellular carcinoma and hepatitis C in the United States, *Hepatology* 36
320 (2002) S74-S83 .
- 321 [6] E.A. Schaefer and R.T. Chung, Anti-hepatitis C virus drugs in development, *Gastroenterology*
322 142 (2012) 1340-1350 .
- 323 [7] U.S. Food and Drug Administration, Incivek (telaprevir) in combination with drugs peginterferon
324 alfa and ribavirin (incivek combination treatment): drug safety communication - serious skin
325 reactions, Electronic Communication, USDA Website (2012) .
- 326 [8] P. Simmonds, Genetic diversity and evolution of hepatitis C virus--15 years on, *J. Gen. Virol.*
327 85 (2004) 3173-3188 .
- 328 [9] C.I. Yu and B.L. Chiang, A new insight into hepatitis C vaccine development, *J. Biomed.*
329 *Biotechnol.* 2010 (2010) 548280 .
- 330 [10] J. Halliday, P. Klenerman, E. Barnes, Vaccination for hepatitis C virus: closing in on an evasive
331 target, *Expert. Rev. Vaccines.* 10 (2011) 659-672 .

- 332 [11] J. Banchereau and R.M. Steinman, Dendritic cells and the control of immunity, *Nature* 392
333 (1998) 245-252 .
- 334 [12] J. Encke, J. Findelee, J. Geib, E. Pfaff, W. Stremmel, Prophylactic and therapeutic
335 vaccination with dendritic cells against hepatitis C virus infection, *Clin. Exp. Immunol.* 142
336 (2005) 362-369 .
- 337 [13] N. Kuzushita, S.H. Gregory, N.A. Monti, R. Carlson, S. Gehring, J.R. Wands, Vaccination with
338 protein-transduced dendritic cells elicits a sustained response to hepatitis C viral antigens,
339 *Gastroenterology* 130 (2006) 453-464 .
- 340 [14] K. Weigand, F. Voigt, J. Encke, B. Hoyler, W. Stremmel, C. Eisenbach, Vaccination with
341 dendritic cells pulsed with hepatitis C pseudo particles induces specific immune responses in
342 mice, *World J. Gastroenterol.* 18 (2012) 785-793 .
- 343 [15] E.J. Gowans, S. Roberts, K. Jones, I. Dinatale, P.A. Latour, B. Chua, E.M. Eriksson, R. Chin,
344 S. Li, D.M. Wall, R.L. Sparrow, J. Moloney, M. Loudovaris, R. Ffrench, H.M. Prince, D. Hart, W.
345 Zeng, J. Torresi, L.E. Brown, D.C. Jackson, A phase I clinical trial of dendritic cell
346 immunotherapy in HCV-infected individuals, *J. Hepatol.* 53 (2010) 599-607 .
- 347 [16] C. Kuiken, K. Yusim, L. Boykin, R. Richardson, The Los Alamos hepatitis C sequence
348 database, *Bioinformatics.* 21 (2005) 379-384 .
- 349 [17] K. Yusim, R. Richardson, N. Tao, A. Dalwani, A. Agrawal, J. Szinger, R. Funkhouser, B.
350 Korber, C. Kuiken, Los Alamos hepatitis C immunology database, *Appl. Bioinformatics.* 4
351 (2005) 217-225 .
- 352 [18] J.R. Schafer, B.M. Jesdale, J.A. George, N.M. Kouttab, A.S. De Groot, Prediction of well-
353 conserved HIV-1 ligands using a matrix-based algorithm, *EpiMatrix*, *Vaccine* 16 (1998) 1880-
354 1884 .
- 355 [19] J.M. Ellis, V. Henson, R. Slack, J. Ng, R.J. Hartzman, H.C. Katovich, Frequencies of HLA-A2
356 alleles in five U.S. population groups. Predominance Of A*02011 and identification of HLA-
357 A*0231, *Hum. Immunol.* 61 (2000) 334-340 .

- 358 [20] S. Southwood, J. Sidney, A. Kondo, M.F. del Guercio, E. Appella, S. Hoffman, R.T. Kubo, R.W.
359 Chesnut, H.M. Grey, A. Sette, Several common HLA-DR types share largely overlapping
360 peptide binding repertoires, *J. Immunol.* 160 (1998) 3363-3373 .
- 361 [21] A.S. De Groot, B.M. Jesdale, E. Szu, J.R. Schafer, R.M. Chicz, G. Deocampo, An interactive
362 Web site providing major histocompatibility ligand predictions: application to HIV research,
363 *AIDS Res. Hum. Retroviruses* 13 (1997) 529-531 .
- 364 [22] A.S. De Groot and W. Martin, Reducing risk, improving outcomes: bioengineering less
365 immunogenic protein therapeutics, *Clin. Immunol.* 131 (2009) 189-201 .
- 366 [23] A.S. De Groot, E.A. Bishop, B. Khan, M. Lally, L. Marcon, J. Franco, K.H. Mayer, C.C.
367 Carpenter, W. Martin, Engineering immunogenic consensus T helper epitopes for a cross-
368 clade HIV vaccine, *Methods* 34 (2004) 476-487 .
- 369 [24] M. Ardito, J. Fueyo, R. Tassone, F. Terry, K. DaSilva, S. Zhang, W. Martin, A.S. De Groot, S.F.
370 Moss, L. Moise, An integrated genomic and immunoinformatic approach to *H. pylori* vaccine
371 design, *Immunome. Res.* 7 (2011) 1 .
- 372 [25] J.A. McMurry, S.H. Gregory, L. Moise, D. Rivera, S. Buus, A.S. De Groot, Diversity of
373 *Francisella tularensis* Schu4 antigens recognized by T lymphocytes after natural infections in
374 humans: Identification of candidate epitopes for inclusion in a rationally designed tularemia
375 vaccine, *Vaccine* 25 (2007) 3179-3191 .
- 376 [26] R. Buchli, R.S. VanGundy, H.D. Hickman-Miller, C.F. Giberson, W. Bardet, W.H. Hildebrand,
377 Development and validation of a fluorescence polarization-based competitive peptide-binding
378 assay for HLA-A*0201--a new tool for epitope discovery, *Biochemistry* 44 (2005) 12491-12507
379 .
- 380 [27] T.P. Meyer, I. Zehnter, B. Hofmann, J. Zaisserer, J. Burkhart, S. Rapp, F. Weinauer, J.
381 Schmitz, W.E. Illert, Filter Buffy Coats (FBC): a source of peripheral blood leukocytes
382 recovered from leukocyte depletion filters, *J. Immunol. Methods* 307 (2005) 150-166 .

- 383 [28] D. Wullner, L. Zhou, E. Bramhall, A. Kuck, T.J. Goletz, S. Swanson, N. Chirmule, V. Jawa,
384 Considerations for optimization and validation of an *in vitro* PBMC derived T cell assay for
385 immunogenicity prediction of biotherapeutics, *Clin. Immunol.* 137 (2010) 5-14 .
- 386 [29] J.M. Moser, E.R. Sassano, d.C. Leistritz, J.M. Eatrides, S. Phogat, W. Koff, D.R. Drake, III,
387 Optimization of a dendritic cell-based assay for the *in vitro* priming of naive human CD4⁺ T
388 cells , *J. Immunol. Methods* 353 (2010) 8-19 .
- 389 [30] M.G. Ghany, D.R. Nelson, D.B. Strader, D.L. Thomas, L.B. Seeff, An update on treatment of
390 genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American
391 Association for the Study of Liver Diseases, *Hepatology* 54 (2011) 1433-1444 .
- 392 [31] A. Tungol, K. Rademacher, J.A. Schafer, Formulary management of the protease inhibitors
393 boceprevir and telaprevir for chronic hepatitis C virus, *J. Manag. Care Pharm.* 17 (2011) 685-
394 694 .
- 395 [32] Department of Health and Human Services, Combating the silent epidemic of viral hepatitis:
396 action plan for the prevention, care & treatment of viral hepatitis, Available at: [http://www.hhs.](http://www.hhs.gov/ash/initiatives/hepatitis/actionplan_viralhepatitis2011.pdf)
397 [gov/ash/initiatives/hepatitis/actionplan_viralhepatitis2011.pdf](http://www.hhs.gov/ash/initiatives/hepatitis/actionplan_viralhepatitis2011.pdf) (2011) .
- 398 [33] G.M. Lauer, Immune responses to hepatitis C virus (HCV) infection and the prospects for an
399 effective HCV vaccine or immunotherapies, *J. Infect. Dis.* 207 Suppl 1 (2013) S7-S12 .
- 400 [34] C.S. Klade, H. Wedemeyer, T. Berg, H. Hinrichsen, G. Cholewinska, S. Zeuzem, H. Blum, M.
401 Buschle, S. Jelovcan, V. Buerger, E. Tauber, J. Frisch, M.P. Manns, Therapeutic vaccination of
402 chronic hepatitis C nonresponder patients with the peptide vaccine IC41, *Gastroenterology* 134
403 (2008) 1385-1395 .
- 404 [35] S. Yutani, A. Yamada, K. Yoshida, Y. Takao, M. Tamura, N. Komatsu, T. Ide, M. Tanaka, M.
405 Sata, K. Itoh, Phase I clinical study of a personalized peptide vaccination for patients infected
406 with hepatitis C virus (HCV) 1b who failed to respond to interferon-based therapy, *Vaccine* 25
407 (2007) 7429-7435 .

408 [36] O. Weiland, G. Ahlen, H. Diepolder, M.C. Jung, S. Levander, M. Fons, I. Mathiesen, N.Y.
409 Sardesai, A. Vahlne, L. Frelin, M. Sallberg, Therapeutic DNA vaccination using *in vivo*
410 electroporation followed by standard of care therapy in patients with genotype 1 chronic
411 hepatitis C, Mol. Ther. 21 (2013) 1796-1805 .

412 [37] F. Habersetzer, G. Honnet, C. Bain, M. Maynard-Muet, V. Leroy, J.P. Zarski, C. Feray, T.F.
413 Baumert, J.P. Bronowicki, M. Doffoel, C. Trepo, D. Agathon, M.L. Toh, M. Baudin, J.Y.
414 Bonnefoy, J.M. Limacher, G. Inchauspe, A poxvirus vaccine is safe, induces T-cell responses,
415 and decreases viral load in patients with chronic hepatitis C, Gastroenterology 141 (2011) 890-
416 899 .

417 [38] K. Palucka and J. Banchereau, Cancer immunotherapy via dendritic cells, Nat. Rev. Cancer 12
418 (2012) 265-277 .

419 [39] L. Levitz, O.A. Koita, K. Sangare, M.T. Ardito, C.M. Boyle, J. Rozehnal, K. Tounkara, S.M.
420 Dao, Y. Kone, Z. Koty, S. Buus, L. Moise, W.D. Martin, A.S. De Groot, Conservation of HIV-1 T
421 cell epitopes across time and clades: validation of immunogenic HLA-A2 epitopes selected for
422 the GAIA HIV vaccine, Vaccine 30 (2012) 7547-7560 .

423 [40] P. Metz, E. Dazert, A. Ruggieri, J. Mazur, L. Kaderali, A. Kaul, U. Zeuge, M.P. Windisch, M.
424 Trippler, V. Lohmann, M. Binder, M. Frese, R. Bartenschlager, Identification of type I and type
425 II interferon-induced effectors controlling hepatitis C virus replication, Hepatology 56 (2012)
426 2082-2093 .

427 [41] J. Jo, U. Aichele, N. Kersting, R. Klein, P. Aichele, E. Bisse, A.K. Sewell, H.E. Blum, R.
428 Bartenschlager, V. Lohmann, R. Thimme, Analysis of CD8⁺ T-cell-mediated inhibition of
429 hepatitis C virus replication using a novel immunological model, Gastroenterology 136 (2009)
430 1391-1401 .

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434 **Table I.** Predicted, HLA-A*0201-restricted HCV epitopes

435	436	Peptide ID#	Name	Sequence	A*0201 Z Score	Conservation	
						1a	1b
437	1	HCV_G1_NS4b_1917	WMNRLIAFA	2.48	100%	99%	
438	2	HCV_G1a_NS5b_2734	MLVCGDDL	2.44	100%	ns ^a	
439	3	HCV_G1_NS4b_1765	HMWNFISGI	2.66	98%	94%	
440	4	HCV_G1_NS3_1451	SVIDCNTCV	2.60	98%	99%	
441	5	HCV_G1b_NS5b_2829	WLGNIIMYA	2.80	ns	97%	
442	6	HCV_G1_E2_615	RLWHYPCTV	3.24	35%	86%	
443	7	HCV_NS3_1586	YLVAYQATV	3.20	99%	86%	
444	8	HCV_G1_E2_685	ALSTGLIHL	2.68	74%	97%	
445	9	HCV_G1_core_133	DLMGYIPLV	2.91	95%	97%	
446	10	HCV_G1_NS3_1074	CINGVCWTV	2.73	92%	34%	
447	11	HCV_G1_E1_323	MMMNWSPTT	2.54	70%	61%	
448	12	HCV_G1_E1_364	SMVGNWAKV	2.42	90%	80%	
449	13	HCV_G1_NS3_1274	GIDPNIRTGV	2.07	56%	16%	
450	14	HCV_G1_NS4b_1808	LLFNILGGWV	2.95	93%	91%	
451	15	HCV_G1_NS3_1607	QMWKCLIRL	2.76	72%	84%	
452	16	HCV_G1_NS5b_2559	IMAKNEVFCV	2.63	98%	75%	
453	17	HCV_G1_E1_277	YVGDLCSV	2.62	91%	92%	
454	18	HCV_G1_E1_281	DLCGSVFLV	2.59	84%	74%	
455	19	HCV_G1_NS5b_2945	YLFNWAVRT	2.57	78%	61%	
456	20	HCV_G1_NS4b_1769	FISGIQYLA	2.51	98%	95%	
457	21	HCV_G1_NS3_1326	SILGIGTVL	2.32	90%	31%	

^ans = not significant.

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459 **Table II.** Predicted, HLA-DRB1-restricted HCV ICS
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461	Peptide	Name	Sequence	DRB1*0101	Predicted #	Conservation	
462	ID#			Z Score	Epitopes	1a	1b
463	1	HCV_G1_NS3_1246	AQGYKVLVLNPSVAATLGFG	2.15	20	>90%	>90%
464	2	HCV_G1_NS4b_1876	VDLLVNLLPAILSPGA	2.75	16	>90%	>90%
465	3	HCV_G1_NS5b_2879	LGNIQRLHGLSAFSLHSY	2.85	15	>90%	>90%
466	4	HCV_G1_NS4b_1769	ISGIQYLAGLSTLPGNPA	2.54	11	>90%	>90%
467	5	HCV_G1_NS4b_1941	AARVTQILSSLTITQLLKRLHQWI	2.33	23	>90%	>80%
468	6	HCV_G1_NS5b_2440	KLPINALSNSLLRHH	3.00	8	>90%	>80%
469	7	HCV_G1_NS4b_1725	AEQFKQKALGLLQTASRQAE	1.76	12	>90%	>80%
470	8	HCV_G1a_NS5b_2485	LQVLKEVAAAASKVKANL	2.14	11	>90%	ns ^a
471	9	HCV_G1_NS4b_1790	LMAFTAAVTSPLTTS	2.55	18	>80%	>80%
472	10	HCV_G1_NS5b_2840	WARMILMTHFFSVLIARDQLEQ	1.96	14	>80%	>80%
473	11	HCV_G1_E2_732	AYCLWMMLLISQAEAALELIT	1.87	16	>80%	>70%
474	12	HCV_G1a_E1_255	AAILRRHIDLLVGSATLCSALY	2.20	13	>80%	ns
475	13	HCV_G1_NS3_1605	DQMWKCLIRLKPTLHGPTP	2.30	15	>70%	>80%
476	14	HCV_G1_NS5b_2941	CGKYLFNWAVRTKLKLT	2.61	11	>70%	>60%
477	15	HCV_G1a_E1_359	GIAYFSMVGWNWAKVL	2.75	5	>70%	ns
478	16	HCV_G1a_NS2_909	VPYFVRVQGLLRICALARKAV	2.58	24	>60%	ns
479	17	HCV_G1b_NS5b_2898	PGEINRVASCLRKLGVPPLRAY	2.31	12	ns	>80%
480	18	HCV_G1b_NS5b_2913	VPPLRVWRHRARSVRAKLLSQGGRA	1.90	16	ns	>70%
481	19	HCV_G1b_NS2_748	LENLVVLNAASVAGAHW	2.54	17	ns	>60%

^ans = not significant.

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486 **Table III.** Predicted, HCV class-I restricted epitopes bind HLA-A*0201

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488	Peptide ID#	Sequence	IC ₅₀
489	1	HCV_G1_NS4b_1917	Black bar
490	2	HCV_G1a_NS5b_2734	Black bar
491	3	HCV_G1_NS4b_1765	Black bar
492	4	HCV_G1_NS3_1451	Black bar
493	5	HCV_G1b_NS5b_2829	Black bar
494	6	HCV_G1_E2_615	Black bar
495	7	HCV_NS3_1586	Gray bar
496	8	HCV_G1_E2_685	Black bar
497	9	HCV_G1_core_133	Black bar
498	10	HCV_G1_NS3_1074	Gray bar
499	11	HCV_G1_E1_323	Black bar
500	12	HCV_G1_E1_364	Black bar
501	13	HCV_G1_NS3_1274	Gray bar
502	14	HCV_G1_NS4b_1808	Gray bar
503	15	HCV_G1_NS3_1607	Black bar
504	16	HCV_G1_NS5b_2559	Black bar
505	17	HCV_G1_E1_277	Gray bar
506	18	HCV_G1_E1_281	Black bar
507	19	HCV_G1_NS5b_2945	Black bar
508	20	HCV_G1_NS4b_1769	White bar
509	21	HCV_G1_NS3_1326	White bar

511 The peptides listed exhibited: IC₅₀ high (IC₅₀<5 μM, black bar);
 512 moderate (5 μM<IC₅₀<50 μM, gray bar) or no (IC₅₀>100 μM; white
 513 bar) affinity for HLA-A*0201 in a competitive binding assay.

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517 **Table IV.** Promiscuous, class II-restricted HCV ICS bind multiple HLA-DRB1 alleles

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519	Peptide ID#	Name	*0101	*0301	*0401	*0701	*1101	*1501
521	1	HCV_G1_NS3_1246	Black	Black	Black	Black	Black	Black
522	2	HCV_G1_NS4b_1876	Black	White	White	White	Black	Black
523	3	HCV_G1_NS5b_2879	Black	Gray	Gray	Black	Black	Black
524	4	HCV_G1_NS4b_1769	Black	White	Black	White	Black	Black
525	5	HCV_G1_NS4b_1941	Gray	Gray	Gray	Gray	Black	Gray
526	6	HCV_G1_NS5b_2440	Black	Gray	White	Gray	White	Black
527	7	HCV_G1_NS4b_1725	White	White	White	Black	Black	Gray
528	8	HCV_G1_NS5b_2485	Gray	Gray	White	Gray	Gray	Gray
529	9	HCV_G1_NS5b_2840	Gray	Black	Black	Black	White	Black
530	10	HCV_G1_NS4b_1790	Black	Black	Black	Black	Gray	Black
531	11	HCV_G1_E2_732	Black	Gray	White	Black	White	Black
532	12	HCV_G1_E1_255	Black	Gray	Gray	Black	Gray	Black
533	13	HCV_G1_NS3_1605	Gray	White	Gray	Gray	Black	Gray
534	14	HCV_G1_NS5b_2941	Black	Gray	Gray	Black	Black	Black
535	15	HCV_G1_E1_359	Black	White	Black	Black	Gray	Black
536	16	HCV_G1_NS2_909	Gray	Gray	Gray	Black	Black	Black
537	17	HCV_G1_NS5b_2898	Black	Gray	White	White	Black	Black
538	18	HCV_G1_NS5b_2913	Gray	White	White	Black	Black	Black
539	19	HCV_G1_NS2_748	Black	Gray	Black	Black	Gray	Black

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 541 The peptides listed exhibited: IC₅₀ high (IC₅₀<10 μM, black bar); moderate (10
 542 μM<IC₅₀<100 μM, gray bar) or low (IC₅₀>100 μM; white bar) affinity for the alleles
 543 denoted in a competitive binding assay.

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544 **Figure Legends**

545 **Figure 1.** Predicted HLA-A*0201- and HLA-DRB1-restricted HCV peptides induce epitope-specific
546 IFN- γ production by naïve human PBMCs. PBMCs back-flushed from whole-blood leukocyte
547 reduction filters were cultured with the HLA-A*0201-restricted (top) or HLA-DRB1-restricted (bottom)
548 peptides listed. The cells were collected after 14 days incubation and IFN- γ ELISpot assays were
549 performed. Each bar represents the average of triplicate wells minus the average negative control
550 (0.1% DMSO + 2 SD) and the analysis of cells obtained from 5 and 9 healthy donors are shown in
551 the top and bottom panels, respectively.

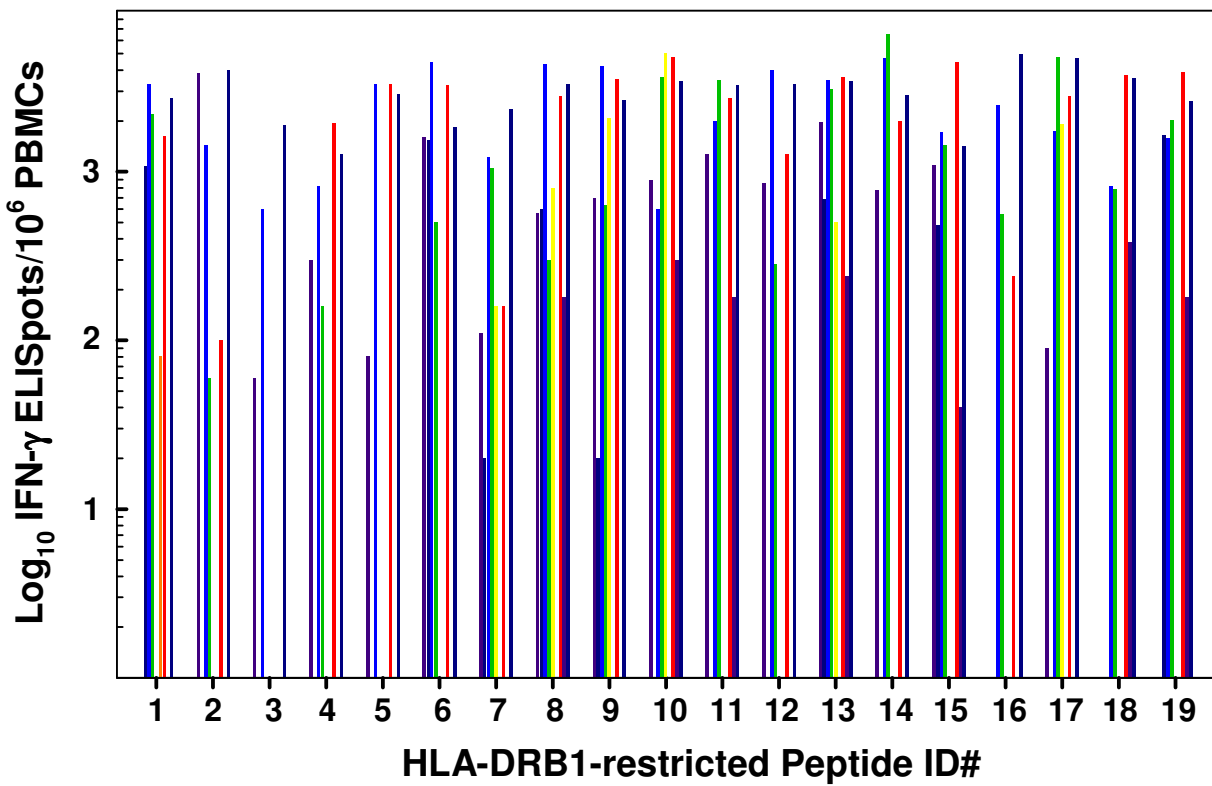
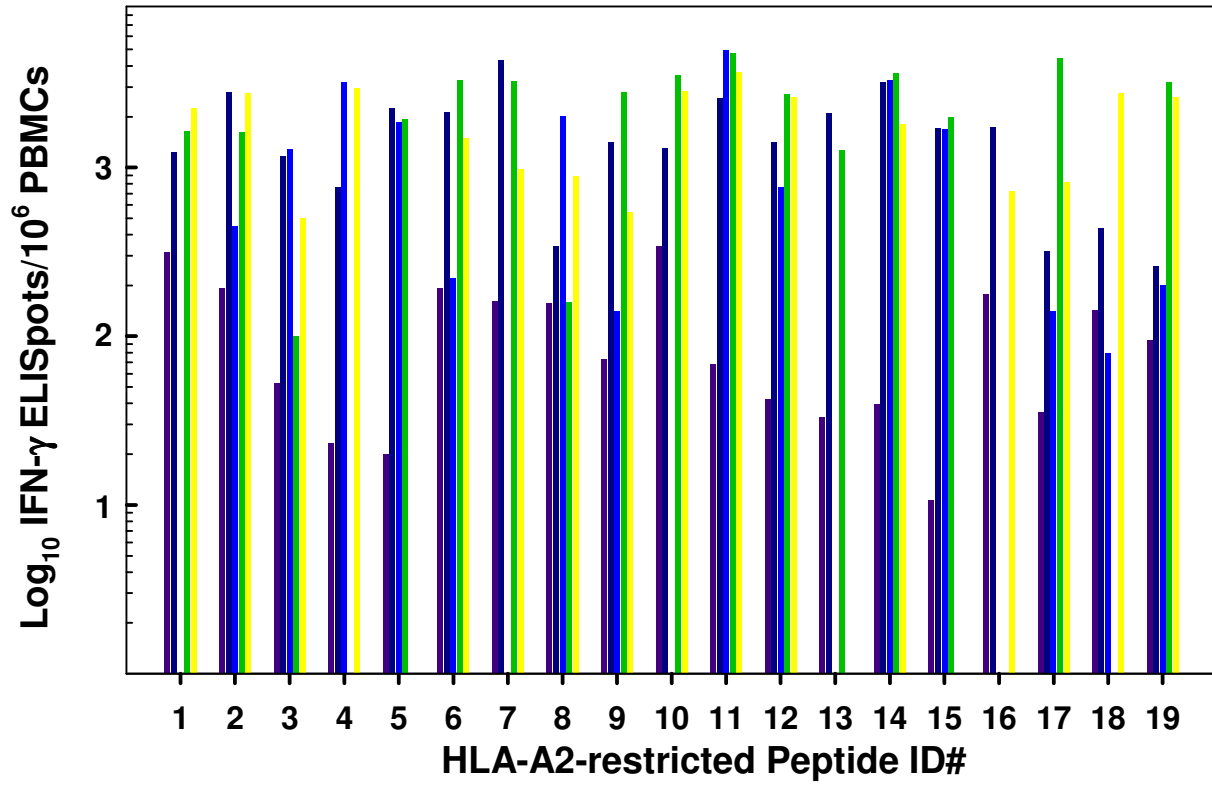
552 **Figure 2.** Peptide-pulsed DCs induce HCV epitope-specific IFN- γ production by naïve human T
553 cells. Purified T cells were co-cultured with monocyte-derived DCs pulsed with the HLA-A2-
554 restricted (top) and HLA-DRB1-restricted (bottom) peptides listed. The sensitized T cells were
555 collected after 14 days and mixed with fresh, peptide-pulsed DCs and IFN- γ ELISpot assays were
556 performed. Each bar represents the average of triplicate wells minus the average negative control
557 (0.1% DMSO + 2 SD) and the analysis of cells obtained from 6 and 8 healthy donors shown in the
558 top and bottom panels, respectively.

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