HCV-Specific T-Cell Immune Responses in Seronegative Injection Drug Users

Marija Zeremski[1], **Marla A. Shu**[1], **Queenie Brown**[1], **Yingfeng Wu**[1], **Don Des Jarlais**[2], **Michael P. Busch**[3,4], **Andrew H. Talal**[1], and **Brian R. Edlin**[1]

[1]Division of Gastroenterology and Hepatology, Dept of Medicine and Center for the Study of Hepatitis C, Weill Cornell Medical College, New York, NY

[2]Baron Edmond de Rothschild Chemical Dependency Institute, Beth Israel Medical Center, New York, NY

[3]Blood Systems Research Institute, San Francisco, CA

[4]University of California, San Francisco, CA

Abstract

Background/Aims—T-cell responses to HCV antigens have been reported in high-risk HCV seronegative persons, suggesting that an effective cellular immune response might be able to clear infection without the development of antibodies. Such findings, however, could be explained by waning antibody or cross-reactivity to other antigens. To address these issues, we assessed T-cell responses in high-risk, seronegative, young IDUs to multiple peptide mixes spanning the entire HCV genome.

Methods—We evaluated HCV-specific T-cell responses in 26 young (age 18-33 years) aviremic, seronegative IDUs (median duration of injection, 6 years) by interferon-γ ELISpot assay using 429 overlapping HCV peptides pooled in 21 mixes. Seventeen aviremic, seropositive IDUs (spontaneous resolvers) and 15 healthy people were used as positive and negative controls, respectively.

Results—The percentage of patients with HCV-specific cellular immune responses was similar in seronegative and seropositive aviremic IDUs (46% versus 59%, p=0.4), while these responses were not detected in any of the negative controls. Among the seronegative IDUs, 6 (23%) had intermediate to very strong responses to 10-20 peptide mixes and another 6 (23%) had moderately strong responses to 2 to 6 mixes. The 12 seronegative IDUs with HCV-specific T-cell responses had higher demographic and behavioral risk profiles than the 14 IDUs without T-cell responses (estimated risk of HCV infection, 0.47 vs. 0.26, p <0.01).

Conclusions—HCV-specific T-cell responses are common among high-risk, seronegative IDUs. The responses are broad and are associated with risk factors for HCV exposure, suggesting that they reflect true exposure to HCV in seronegative persons.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease that affects >120 million people worldwide and nearly 5 million people in the United States (1-3). Injection drug use is currently the primary mode of HCV transmission in the developed world (4).

Address reprints and correspondence: Andrew Talal, M.D., M.P:H., Weill Cornell Medical College, 525 E. 68th Street, Box 319, New York, NY 10065-4896, Phone: 212-746-4495, FAX: 212-746-7977, aht2002@med.cornell.edu.

HCV seroprevalence in long-term injection drug users (IDUs) ranges from 60% to > 90% (5-8). Since the introduction of needle exchange programs and other interventions designed to control bloodborne virus transmission among IDUs, however, HCV seroprevalence estimates in IDUs who have injected drugs for less than 5 years are generally less than 50% (9-12). Nevertheless, incidence rates among uninfected IDUs remain extremely high, ranging from 10% to 40% per year (13-16).

IDUs who have successfully cleared a previous HCV infection, however, have a reduced risk of subsequently developing persistent HCV viremia even if they continue injection drug use (17,18), suggesting that some IDUs possess immunity that confers at least partial protection from subsequent infection. Viral clearance may even occur in individuals who do not seroconvert. Several groups, for example, have reported cellular immune responses to HCV antigens in antibody-negative persons who may have been exposed to the virus, including healthcare workers (19), spouses (20), and other family members of persons with HCV infection (21). In prison inmates, another high-risk group, viral clearance has been associated with cellular immunity in the absence of seroconversion (22). These findings suggest that cellular immune responses alone may be capable of clearing HCV infection without the development of antibodies.

Cellular immune responses to HCV antigens have been reported in high-risk seronegative IDUs (23,24). Such findings, however, could be explained by seroreversion (25) or cross-reactivity to other antigens (26). Seroreversion may occur in a substantial proportion of individuals 10 years or more after they clear HCV infection spontaneously. In a study of women who spontaneously cleared HCV infection acquired through contaminated human Rh immunoglobulin, antibody responses were present in 10 of 10 women tested 10 years after exposure but absent in 18 (42%) of 43 women tested 18-20 years after exposure (25). In an IDU study, Mizukoshi *et al*. reported T-cell responses to multiple HCV antigens in seronegative IDUs in the San Francisco Bay area, but seroreversion could not be excluded because the median age of the IDUs was 44.5 years, and the median duration of injection, 18 years (24). HCV-specific T-cell responses may also be detected in healthy, uninfected persons because of cross-reactivity between an immunodominant epitope in the HCV NS3 protein and the influenza virus neuraminidase protein (26). Other antigens may also cross-react with HCV proteins, and cross-reactivity may be particularly common among IDUs, who are repeatedly exposed to many foreign antigens through nonsterile drug injection. In a second IDU study, Freeman *et al*. reported cellular immune responses to two HCV polyprotein constructs in seronegative IDUs (23). Approximately one third of the IDUs with positive responses, however, responded only to the construct that included the NS3 region.

In the present study, we evaluated HCV-specific cellular responses in a sample of young aviremic, seronegative IDUs with a relatively short duration of injection drug use recruited in community settings. We studied young IDUs with a short injection history to exclude the possibility of seroreversion in seronegative individuals with T-cell responses. We assessed the breadth of HCV-specific cellular immune responses by measuring the reactivity to 21 peptide mixes encompassing the entire HCV genome. To further assess whether these responses likely resulted from exposure to HCV, we examined the association between behaviors that might facilitate HCV exposure and the presence of HCV-specific cellular responses.

Materials and Methods

Subjects characteristics

Subjects were selected from the Swan Study, which recruited IDUs in Manhattan through a needle exchange program, street outreach, and word-of-mouth. Eligibility criteria were age

18 to 35 years and injection of illicit drugs at least once in the 30 days before enrollment. At enrollment, interviewers administered a standardized questionnaire and blood was drawn for HCV and HIV antibody and HCV RNA testing. HCV antibodies were assayed by second (HCV EIA 2.0, Abbott Laboratories, Abbott Park, IL) and third (HCV 3.0 ELISA and RIBA HCV 3.0, Ortho Clinical Diagnostics, Raritan, NJ) generation testing. The presence of HCV RNA was determined with the discriminatory HCV transcription-mediated amplification (dHCV TMA) assay (Procleix HIV-1/HCV, Chiron, Emeryville, CA (27)), with the lower limit of detection of 100 copies/mL 100% of the time, 10 copies/mL 75% of the time, and 3 copies/mL 40% of the time (each concentration tested with 60 replicates). Aviremic, antibody-negative subjects were offered enrollment in a longitudinal study in which HCV RNA testing was performed biweekly. The protocol was approved by the Weill Cornell Medical College Institutional Review Board and was consistent with the standards established by the Helsinki Declaration of 1975. Written informed consent was obtained from all subjects.

Of 270 IDUs enrolled and tested in the Swan Study from February 2005 through July 2007, PBMCs were available from 26 HCV antibody-negative and 17 HCV antibody-positive participants who repeatedly tested negative for HCV RNA. The 26 HCV antibody-negative IDUs had negative HCV RNA tests on a median of 11 (interquartile range [IQR], 6-16) samples drawn a median of 24 (IQR, 19-40) weeks after the PBMCs were collected. They did not significantly differ from the 126 HCV antibody-negative IDUs who were not included in the present study in age, sex, ethnicity, injection duration, or injection practices. The HCV antibody-positive, RNA-negative IDUs, presumed spontaneous resolvers, were included as positive controls. Fifteen healthy individuals without risk factors for HCV infection were included as negative controls.

Samples

Blood samples were kept at room temperature before separation. PBMCs were isolated using Ficoll-Hypaque density gradient centrifugation within 24 hours of specimen collection and cryopreserved at -150°C.

HCV antigens

A total of 429 18-mer peptides, each overlapping the next by 11 amino acids, which encompass the complete HCV genotype 1a (strain H77) polyprotein, were obtained from NIH AIDS Research & Reference Reagent Program (McKesson BioServices Corporation, Germantown, MD). Peptides were resuspended at 20 mg/ml in dimethyl sulfoxide and were further diluted to 1 mg/ml by adding phosphate-buffered saline (PBS). Peptides were pooled into 21 mixtures (A to U), 20 mixtures containing 21 peptides and one (mixture U) containing 9 peptides.

IFN-γ enzyme-linked immunospot (ELISpot) assay

Ninety-six-well plates (Millipore, Bedford, MA) were coated overnight at 4°C with the primary IFN-γ antibody (mAb 1-D1K, MABTECH, Cincinnati, OH) diluted in sterile PBS to a final concentration of 5 μg/ml. Subsequently, plates were washed 4 times with sterile PBS, blocked for one hour at room temperature with 1% bovine serum albumin (BSA) in PBS, washed 2-3 times with sterile PBS, and blocked for another 30-60 min with cell medium (RPMI medium, 5% human AB serum, and 2 mM L-glutamine). Cryopreserved PBMCs were thawed and plated at 300,000 cells/well in cell medium. Each peptide mix, with individual HCV peptides at a concentration of 2 μg/ml, was added to three replicate wells. Phorbol mystriate acetate in a final concentration of 5 ng/ml, tetanus toxoid (Accurate Chemicals & Scientific Corporation, Westbury, NY) at a final concentration of 5 μg/ml, and Candida albicans (Greer, Lenoir, NC) cellular antigen at a final concentration 10 μg/ml were

used as positive controls; all controls were also plated in triplicate. Each plate had at least 6 no-antigen control wells.

After 40 hours of incubation, plates were washed 3 times with PBS, 4 times with PBS/ 0.01% Tween 20 and then incubated for 2 hours with 1 μg/ml secondary IFN-γ biotinylated antibody (mAb 7-B6-1-Biotin, MABTECH). After rinsing with PBS/0.01% Tween 20 and a one hour incubation with strepatavidin-alkaline phosphatase (Vectastain ABC kit, Vector Laboratories, Burlingame, CA), plates were washed again 3 times with PBS/0.01% Tween 20, 3 times with PBS, and developed using AEC Chromagen/Substrate kit (DBS, Pleasanton, CA). The reaction was stopped by rinsing plates with water. Spots were counted with a KS ELISpot reader (Zeiss, Thornwood, NY). All no-antigen (background) control wells had fewer than 10 spot-forming cells (SFC) per well. Responses were considered positive if, after subtraction of the background, the mean number of SFC was at least 15 and at least the mean + 3 standard deviations of the healthy controls for the respective peptide mix. Responses were classified according to the number of SFC per well over the higher threshold as follows: weak - 1 to 10 SFC, intermediate – 11 to 49 SFC, strong – 50 to 100 SFC, and very strong >100 SFC. Patients were considered positive for HCV-specific cellular responses if more than one peptide mix was positive according to the above criteria.

Statistical analysis

To determine whether positive T-cell responses were associated with exposure to HCV, we first identified indicators of exposure by examining the associations between demographic and behavioral factors and HCV antibody in the 270 eligible IDUs participating in the Swan Study. Eleven demographic and behavioral factors (listed in Table 3) were significantly associated with a higher likelihood of having HCV antibody in univariate analyses using chi-square tests for binary variables and Mantel-Haantzel chi-square tests for trend for ordinal variables. We then examined these 11 factors in the seronegative IDUs, comparing those who had T-cell responses with those who did not. Next, we fitted a multiple logistic regression model to determine independent risk factors for HCV antibody in the 270 eligible study participants. Six of the eleven risk factors were found to independently predict the risk of having HCV antibody (Table 4). The risk estimates for each of these six factors generated by this model were then applied to the demographic and behavioral data from each of the 26 seronegative IDUs in the present study to estimate the expected likelihood of HCV infection for each of them (Table 5). Even though the IDUs in this study were seronegative, the model predicted their risk of having HCV antibody based on the six factors found to predict HCV antibody in the entire cohort. We then compared the predicted risks of HCV infection derived from these analyses in the seronegative IDUs with and without HCV-specific T-cell responses, using Student's t-test. All statistical analyses were conducted using Statistical Analysis Software (SAS Institute, Cary, N.C.).

Results

Study subjects

Among the 43 aviremic IDUs included in this study (26 HCV seronegative subjects and 17 seropositive controls), the median duration of injection drug use was 7 (range, 1-18) years. Most participants were Caucasian, and their median age was 26 (range, 18-33) years (Table 1). High-risk behaviors for acquisition of blood-borne infections were frequently reported by the members of this cohort. Nearly all (95%) had injected heroin and nearly half (47%) had injected cocaine in the 30 days preceding study enrollment. They had injected drugs frequently (median of 20 heroin injections during the same time period), about half (42%) had injected with syringes previously used by another IDU, and many (30%) had injected drug solution withdrawn from a cooker after another IDU had inserted a used syringe into it.

T-cell responses in IDUs

In order to determine the presence of HCV-specific cellular immune responses, we performed the IFN-γ ELISpot assay using 21 overlapping peptide mixes as antigens. Among the 26 HCV antibody and HCV RNA negative individuals, 12 (46%) had HCV-specific, IFN-γ cellular responses to more than one HCV peptide mix (Fig 1A). There was marked heterogeneity in the strength and breadth of these responses. Six of these subjects (23% of all seronegative IDUs) had intermediate to very strong responses to 10-20 of the antigen mixes (Table 2). Of the remaining 6 subjects, 2 mounted intermediate responses to 6 antigen mixes, 2 subjects had intermediate to strong responses to 4 peptide mixes and 2 subjects weak to intermediate responses to 2 peptide mixes. Of the 17 spontaneous resolvers included as positive controls, 10 (59%) had detectable IFN-γ responses to more than one HCV peptide mix (Fig 1B), which was similar to that observed among the study subjects (46% versus 59%, p = 0.4). These subjects likewise demonstrated marked heterogeneity in the strength and breadth of their responses. Eight of them (47% of all spontaneous resolvers) had intermediate to very strong responses to 8-20 of the antigen mixes. The remaining 2 patients had weak to intermediate responses to 2-3 antigen combinations. Overall, HCV-specific cellular immune responses did not differ significantly in frequency or strength between seronegative and seropositive aviremic IDUs (p = 0.19, Table 2). Of 15 healthy controls, 3 (20%) had weak reactivity to one antigen mix; none met the criteria for HCV-specific cellular responses (Fig 1C).

In our study subjects, immune responses were observed to 20 of the 21-peptide pools, suggesting that T-cell responses were generated to a broad, diverse array of epitopes in nearly every region of the HCV polyprotein (Fig 2). The strongest and most frequent responses were observed toward peptide mixes C and D, which are part of the viral envelope glycoproteins 1 and 2 (Figs 1 and 2). Marked responses were also detected toward peptide mixes M, N, O, R and S, which are part of the nonstructural proteins NS4B, NS5A and NS5B. The least immunogenic response was directed toward the peptide mix U that corresponds to C-terminal part of NS5B (Fig 1 and 2). Two subjects (SN11, SN12) had responses exclusively to peptides corresponding to structural proteins.

Associations between demographic and behavioral factors with T-cell responses

Among the 270 eligible IDUs participating in the Swan Study, the 11 demographic and behavioral factors listed in Table 3 were significantly associated with HCV antibody in univariate analysis (data not shown). In the 26 seronegative IDUs included in this study, 3 of these factors were significantly associated with HCV-specific T-cell responses, and another 5 of the factors had associations with T-cell responses that were in the expected direction and of similar magnitude as their association with HCV antibody in the larger study sample (Table 3). Thus, most of the demographic and behavioral factors associated with having HCV-specific antibodies among all 270 IDUs in the Swan Study also had associations of similar magnitude and direction with having HCV-specific T-cell responses among the 26 seronegative IDUs in the present study. In contrast, when we examined these relationships in the 17 seropositive spontaneous resolvers, T-cell responses were not associated with any of the risk factors.

Six of the risk factors were independently associated with HCV antibody in multivariate analysis among the 270 eligible Swan Study participants (Table 4). The effect estimates from this model were then applied to generate estimates of the predicted risk of HCV infection for each of the 26 HCV antibody-negative IDUs in the present study based on their data on these six variables. These predicted risk estimates, representing a summary measure of the demographic and behavioral indicators of exposure to HCV for each of the seronegative IDUs in the present study, were significantly higher in the 12 seronegative

IDUs who demonstrated HCV-specific T-cell responses than in the 14 without such responses (Table 5).

Discussion

In this study of 26 young, aviremic, seronegative IDUs recruited in New York City, 46% had HCV-specific, IFN-γ cellular responses that suggested prior exposure to HCV. Nineteen (73%) had been injecting for 10 years or less, making seroreversion an unlikely explanation for these findings. The responses were more common in IDUs with demographic and behavioral risk factors for HCV infection, which supports the hypothesis that they were acquired through exposure to HCV. In addition, the detection of a median of 8 responses per subject suggests that they likely represent true immune reactivity to HCV rather than cross-reactivity to other antigens.

Other studies have also detected HCV-specific T-cell responses in seronegative persons at high-risk for viral contact (19-23). Some of these individuals might have had acute HCV infection that spontaneously cleared without seroconvesion (22,28,29). Alternative explanations for seronegativity in spontaneous resolvers are delayed development of antibodies (30,31) or their loss over time (25). Finally, in some high-risk individuals, repeated low-level exposure to HCV may promote the development of cellular immune responses without the development of persistent viremia or serologic responses.

Two previous studies have evaluated cellular immune responses to HCV in IDUs. In one, using whole proteins as antigens, HCV specific T-cell responses were detected in 72% of seronegative IDUs (median age, 27 years; median duration of injection drug use, 7 years) recruited from an Australian primary health care facility (23). In the other, 62% of older seronegative IDUs (median age, 44.5 years; median duration of injection, 18 years) recruited from street settings in the San Francisco Bay area had T-cell responses detected by ELISpot and T-cell proliferation assays using HCV peptides and proteins as antigens (24). Although the seronegative IDUs in the present study were younger (median age, 26 years) and had a shorter duration of injection (median, 6 years), cellular immune responses were still detected in a similar (and statistically indistinguishable) proportion—suggesting that seroreversion was not the explanation. In the San Francisco and the present study, subjects were recruited in community settings to avoid the bias inherent in recruiting IDUs in health care facilities. Taken together, these data suggest that HCV-specific T-cell responses suggesting prior HCV exposure are common among HCV seronegative IDUs, regardless of age, injection duration, or geographic region.

Interestingly, of 17 seropositive individuals who were included in the study as positive controls, only 10 (59%) had positive T-cell responses. This observation suggests that ELISpot assays, like HCV antibodies, may not identify all patients who have mounted effective immune responses to HCV. In these patients, viral clearance may have been achieved through immune responses not detected by IFN-γ ELISpot assays. Alternatively, these responses may have waned over time. In either case, this finding suggests that additional seronegative IDUs may have developed HCV-specific immune responses that were not detected by ELISpot. Consequently, neither cellular nor serological responses may detect all persons with previous HCV exposure. Considering that the overall HCV seroprevalence in the Swan Study was 44% and that we detected HCV-specific T-cell responses in 46% of seronegative IDUs, at least 70% of study participants may have had prior exposure to HCV.

Because peptides were pooled for the ELISpot assay, we do not know the exact epitopes against which T-cell responses were directed. However, certain regions of the genome

appear to be more immunogenic. For example, we detected strong responses against peptide mixes C and D in the majority of the patients. These antigen combinations correspond to the C-terminal of the HCV E1 glycoprotein and the N-terminal of the E2 glycoprotein. Marked responses were also detected toward the peptides that correspond to nonstructural proteins, NS4B, NS5A and NS5B. Exposure to intact viral particles alone in the absence of transcription and translation of viral proteins would not be expected to result in immune reactivity to nonstructural proteins. This suggests that the responses we detected likely resulted from productive HCV infection. Two of our patients, however, exhibited reactivity to structural antigens only, and we cannot exclude exposure to viral particles or replicating virus that does not result in productive infection as a cause of these responses.

In summary we found that a substantial proportion of our aviremic, seronegative IDUs had detectable HCV-specific IFN-γ T-cell responses indicating prior HCV exposure. Prospective studies are needed to determine whether such responses are protective against future infection. If they are, they will have important implications for vaccine development.

Acknowledgments

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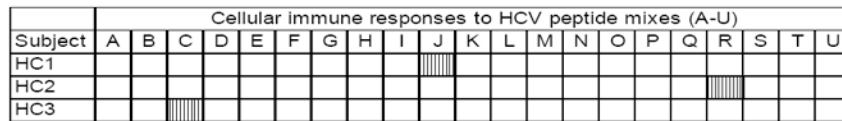
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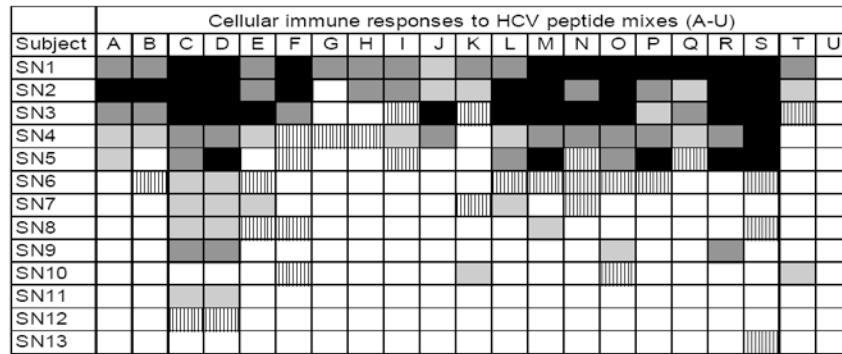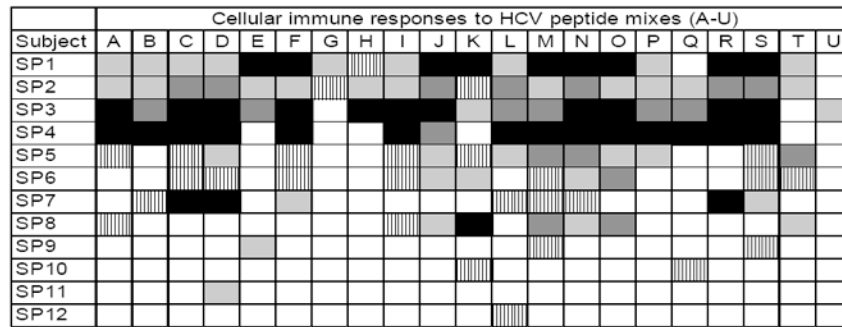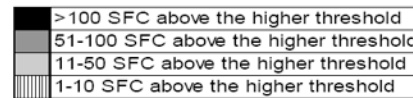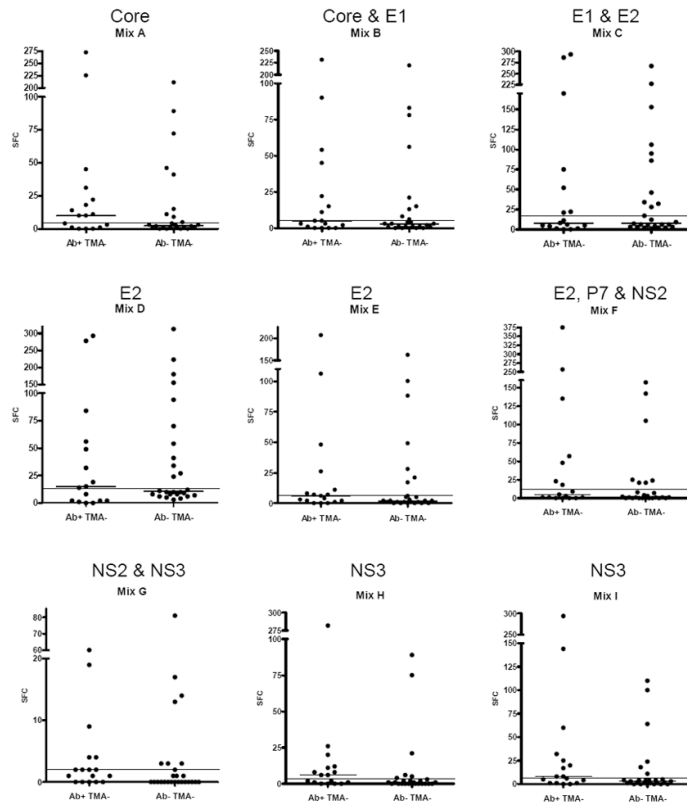
Figure 1.
Strength and breadth of hepatitis C virus (HCV)-specific interferon (IFN)-γ responses among A) aviremic, seronegative injection drug users (IDUs), B) aviremic, seropositive IDUs, and C) healthy controls as determined by enzyme-linked immunospot assay. The response was assessed based upon the number of IFN-γ secreting cells per 3×10^5 peripheral blood mononuclear cells after exposure to 21 peptide mixes covering the entire viral polyprotein. After subtraction of the background, a reaction to each mix was considered positive if it had at least 15 spot forming cells (SFC) and at least the mean number of SFC in the healthy controls + 3 standard deviations. Each mix was graded as to the number of SFC observed over the higher threshold: vertical line pattern indicates 1 to 10 SFC, light gray indicates 11 to 50 SFC, dark gray indicates 51 to 100 SFC, and black indicates > 100 SFC. Of 26 analyzed aviremic, seronegative IDUs, 12 had responses to more than 1 mix and were considered positive for HCV-specific cellular responses. Of 17 aviremic, seropositive IDUs, 10 had responses to more than 1 mix and were considered positive for HCV-specific cellular responses. Of 15 normal controls, only 3 had weak responses to only one peptide mix each and none were considered positive for HCV-specific cellular responses. Subjects that had no responses to any peptide mixes are not shown.
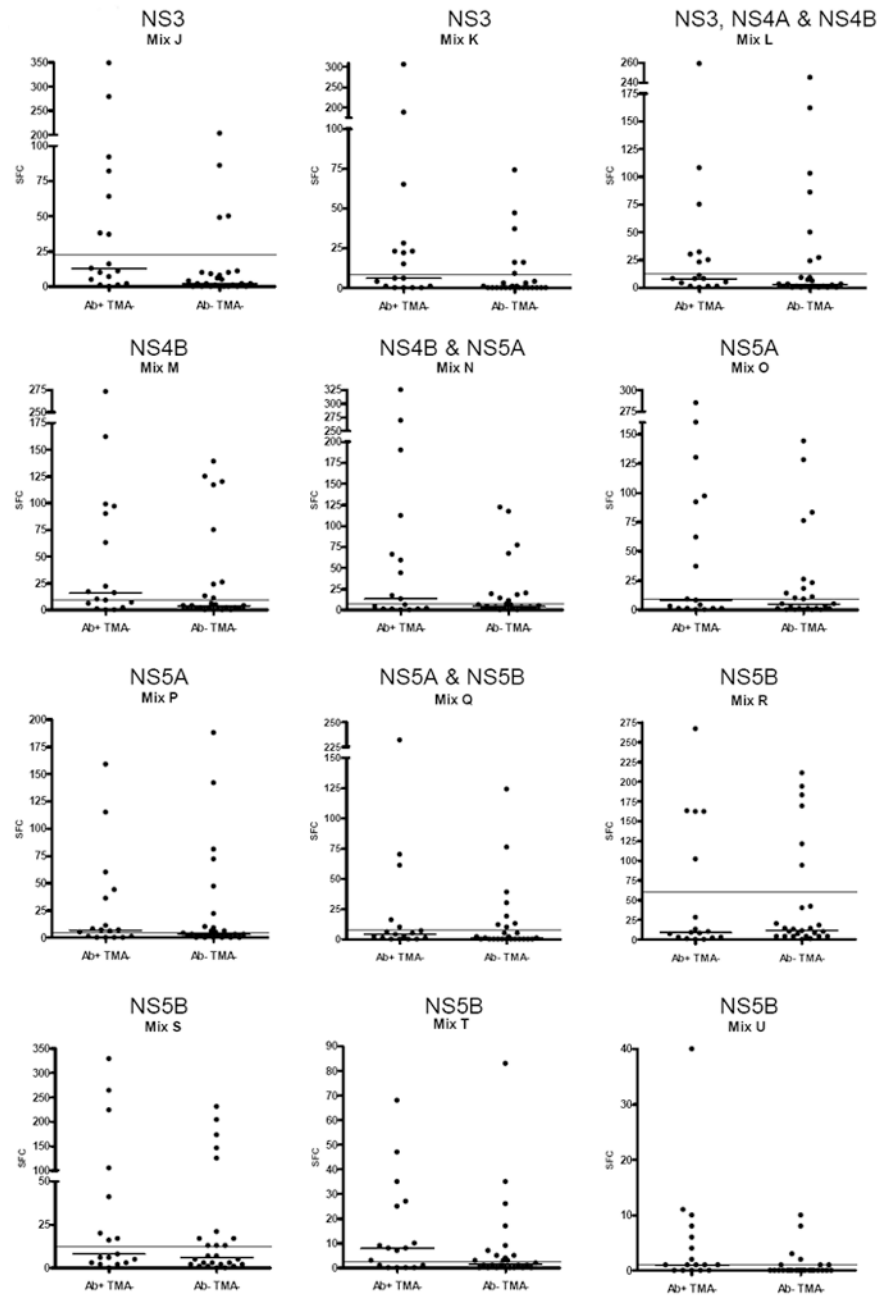
Figure 2.
Number of hepatitis C virus (HCV)-specific interferon (IFN)-γ secreting cells in aviremic, seropositive and aviremic, seronegative individuals after incubation with 21 peptide mixes encompassing the entire viral polyprotein. The corresponding viral proteins are indicated above each mix. Each point indicates the mean number of spot-forming cells (SFC) per well for one subject after subtraction of the background. The short horizontal line indicates the mean for each group of subjects. The long horizontal line shows the mean number of SFC + 3 standard deviations in healthy controls. Note differing Y-axis values in each panel.

Table 1

Characteristics of Seronegative and Seropositive IDUs and Healthy Controls

	HCV RNA Negative IDUs			Healthy Controls (N=15)
	HCV Ab (−) (N=26)	HCV Ab (+) (N=17)	Total IDUs (N=43)	
Age at sample collection, Median *(IQR)*	26 (22,30)	27 (24,30)	26 (22.5,30)	32 (28,39)
Gender, *N (%)*				
Male	15 (58)	12 (71)	27 (63)	6 (40)
Female	10 (38)	4 (24)	14 (33)	9 (60)
Transgender (M-to-F)	1 (4)	1 (6)	2 (5)	0 (0)
Ethnicity, *N (%)*				
White	16 (62)	9 (53)	25 (58)	12 (80)
Black	1 (4)	0 (0)	1 (2)	2 (13)
Hispanic	5 (19)	5 (29)	10 (23)	0 (0)
Other	4 (15)	3 (18)	7 (16)	1 (7)
Duration of injecting (yrs), *Median (IQR)*	6 (4,10)	9 (7,10)	7 (4,10)	0 (0,0)
Frequency of injection, last 30 days (no.of times), *Median (IQR)*	38 (8, 80)	50 (17, 114)	41 (15,91)	0 (0,0)
Drugs injected, last 30 days, *N(%)*				
Heroin	25 (96)	16 (94)	41 (95)	0 (0,0)
Cocaine	11 (42)	9 (53)	20 (47)	0 (0,0)
Crystal methemphetamine or speed	0 (0)	0 (0)	0 (0)	0 (0,0)

Abbreviations: IDUs, injection drug users; HCV, hepatitis C virus; IQR, interquartile range; Ab, antibody.

Table 2

Breadth of HCV-specific, interferon –γ responses among seronegative and seropositive IDUs and healthy controls[*]

No. positive antigen mixes	HCV RNA Negative IDUs		Healthy Controls
	HCV Ab (-)	HCV Ab (+) Controls	Healthy Controls
8-20	6 (23%)	8 (47%)	0
2-6	6 (23%)	2 (12%)	0
0-1	14 (54%)	7 (41%)	15 (100%)

[*] Seronegative and seropositive aviremic IDUs did not differ significantly in the number of antigen mixes to which they demonstrated HCV-specific cellular immune responses (p=0.19, Mantel-Haenszel chi-square test for linear trend).

Abbreviations: HCV, hepatitis C virus; IDUs, injection drug users; Ab, antibody

Table 3

Demographic and behavioral risk factors[*] among seronegative IDUs according to presence or absence of HCV-specific T-cell responses

Characteristic	T-Cell (+)	T-Cell (-)	OR (95% CI)[†]	p[†]
	N (%)	*N (%)*		
Total	12 (46)	14 (54)		
Age (years)[§]				
18-25	4 (33)	8 (57)	1.0	0.22
26-33	8 (67)	6 (43)	2.67 (0.54, 13.2)	
Duration of injection drug use				
0-6 years	6 (50)	7 (50)	1.0	1.00
7-18 years	6 (50)	7 (50)	1.0 (0.16, 6.17)	
Frequency of injection (past 30 days)[§]				
≤ 15 times (less often than every other day)	3 (33)	6 (43)	1.0	0.43
> 15 times	9 (53)	8 (57)	2.25 (0.42, 12.1)	
Ever injected cocaine (or crack)[§]				
No	2 (17)	6 (43)	1.0	0.22
Yes	10 (83)	8 (57)	3.75 (0.59, 23.9)	
# times used a syringe previously used by another IDU (past 6 months)				
None	6 (50)	6 (43)	1.0	
1-9 times	2 (17)	5 (36)	0.4 (0.06, 2.93)	0.88
10 or more times	4 (33)	3 (21)	1.33 (0.20, 8.71)	
# times divided drugs (to share them) with a used syringe (past 6 months)[‡]				
Never	6 (50)	12 (86)	1.0	0.01
1-3 times	1 (8)	2 (14)	1.0 (0.01, 23.1)	
4 or more times	5 (42)	0 (0)	∞ (1.27, ∞)	
# people who had used syringes used to split drugs (past 6 months)[‡]				
None	6 (50)	12 (86)	1.0	
1-3 people	5 (42)	2 (14)	5.0 (0.56, 53.9)	0.04
4-9 people	1 (8)	0	∞ (0.04, ∞)	
# times used cooker after another IDU stuck a used needle in it (past 6 months)[§]				
Never	6 (50)	10 (77)	1.0	
1-3 times	1 (8)	2 (15)	1.2 (0.09, 16.2)	0.07
4 or more times	5 (42)	1 (8)	10.0 (0.4, 250)	
Any injection in shooting gallery (past 6 months)				
No	10 (83)	12 (86)	1.0	1.00
Yes	2 (17)	2 (14)	1.2 (0.14, 10.1)	
Most common location of injection (past 6 months)[§]				
Home[¶]	4 (33)	8 (57)	0.35 (0.08, 1.89)	0.43
Other places	8 (67)	6 (43)	1.0	
Who gave first injection[‡]				

Characteristic	T-Cell (+)	T-Cell (-)	OR (95% CI)†	p†
Self	1 (8)	5 (38)	1.0	
Someone < 30 years old	7 (58)	8 (62)	4.38 (0.33, 236)	0.01
Someone 30 years or older**	4 (33)	0 (0)	∞ (1.02, ∞)	

*
Each of the 11 factors in this table was significantly associated with an increased likelihood of having HCV antibody among the 270 IDUs who participated in the Swan Study.

†Odds ratios and *P* values reflect the association between the characteristic and the presence or absence of HCV-specific T-cell responses (comparing the 12 seronegative IDUs with T-cell responses to the 14 without them).

‡Factors significantly associated with HCV-specific, interferon-γ T-cell responses

§Factors with associations with HCV-specific, interferon-γ T-cell responses that were not significant but are of expected direction and magnitude.

¶Injections in the home allow more opportunity for the use of safer injection techniques.

**
IDUs > 30 years old are more likely to have HCV infection than those younger.

Abbreviations: HCV, hepatitis C virus; IDUs, injection drug users; OR, odds ratio; CI, confidence interval.

Table 4

Factors associated with HCV antibody in multiple logistic regression, IDUs participating in the Swan Study (N=270)

Characteristic	Adjusted OR[*] (95% CI)	p
Age (in years)	1.09 (1.00, 1.19)	0.04
Duration of injection drug use (in years)	1.11 (1.02, 1.22)	0.02
Frequency of injection (past 30 days)		
< 15 times	1	0.03
≥ 15 times	2.22 (1.08, 4.55)	
Ever injected cocaine/crack	3.60 (1.63, 7.93)	<0.01
Most common location of injection (past 6 months)		
Home	0.29 (0.14, 0.57)	<0.01
Another place	1	
Who gave first injection		
Self	1	
Someone < 30 years old	1.16 (0.57, 2.33)	<0.01
Someone ≥ 30 years old	4.59 (1.70, 12.4)	

[*] Adjusted odds ratios and *P* values were calculated to examine factors independently associated with HCV antibody positivity among all 270 participants in the Swan Study.

Abbreviations: HCV, hepatitis C virus; IDUs, injection drug users; OR, odds ratio; CI, confidence interval.

Table 5

Predicted Mean Probability of HCV Infection of HCV Antibody-Negative IDUs by T-Cell Response (N=26)[*]

| T-cell response | N | Predicted probability of HCV infection | | p |
		Mean	(95% CI)	
Negative	14	0.26	(0.14, 0.38)	
Positive	12	0.47	(0.31, 0.62)	<0.01
All patients	26	0.36	(0.26, 0.46)	

[*] Probabilities based on the IDUs' reported demographic and behavioral characteristics and the risk estimates generated by the multivariate model presented in Table 4.

Abbreviations: HCV, hepatitis C virus; IDUs, injection drug users; CI, confidence interval.