JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2012, **19**, 554–559 doi:10.1111/j.1365-2893.2011.01573.x

Hepatitis C virus-specific immune responses in noninjecting drug users

M. Zeremski,[1] J. Makeyeva,[1] K. Arasteh,[2] D. C. Des Jarlais[2] and A. H. Talal[1] [1]*Center for the Study of Hepatitis C, Division of Gastroenterology and Hepatology, Weill Cornell Medical College, New York, NY, USA; and* [2]*Baron Edmond de Rothschild Chemical Dependency Institute, Beth Israel Medical Center, New York, NY, USA*

Received August 2011; accepted for publication November 2011

SUMMARY. Noninjection drug use, although recognized as an emerging risk factor for acquisition of other blood-born pathogens, is still unconfirmed as a route of hepatitis C virus (HCV) transmission. Our goal was to measure HCV exposure and prevalence in noninjection drug users (NIDUs). Fifty-seven NIDUs were screened by extensive questionnaire to exclude prior injection drug use and evaluated for HCV-specific serologic and cellular immune responses. HCV-specific T-cell responses were measured using interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot) assay with overlapping HCV peptides covering the entire HCV genome. Fifteen individuals who never used illicit drugs served as negative controls. Eleven people with no history of injecting drug use (19.3%) were HCV seropositive: seven with chronic HCV infection and four with previously resolved infection. Of 51 NIDUs with ELISpot results, HCV-specific cellular immunity was detected in 5 (9.8%). These responses were relatively weak and narrow. We did not find significant associations between HCV-specific immune responses and noninjection drug use practices. Subjects with HCV-specific immunity, however, were significantly more likely to have bought sex in the past 6 months, to have had more casual partners of the opposite sex in the last 6 months, and those partners were more likely to have ever injected drugs compared to subjects without HCV-specific immunity. In summary, we found serologic or cellular HCV-specific immune responses in 27.5% of NIDUs. Our results suggest that sexual behaviour associated with noninjection drug use might be a risk factor for HCV acquisition. Additional studies are needed to precisely determine the practices that lead to HCV exposure among this population.

Keywords: antibody, cellular immunity, ELISpot, serological immunity.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global health problem with >120 million people affected worldwide and >5 million people in the United States [1–3]. Although some patients spontaneously resolve HCV infection, the majority develops chronic liver disease that can progress to cirrhosis and/or hepatocellular carcinoma. Currently, injection drug use represents the primary mode of HCV transmission in developed countries with >80% prevalence among older injection drug users (IDUs) and incidence rates ranging from 10% to >20% annually [4–10].

Although still controversial, noninjection drug use is recognized as an emerging risk factor for HCV acquisition. HCV prevalence among noninjection drug users (NIDUs) ranges from 2% to 35%, significantly greater than that observed in the general population [11]. Although the mechanism of HCV transmission among NIDUs is incompletely understood, sharing of equipment used for administration of illicit drugs is the most plausible explanation [12]. HCV RNA has been detected in saliva [13,14], gingival crevicular fluid [14], and nasal secretions [15] in HCV-infected people. Additionally, vasoconstricting effects or irritation caused by illicit drugs, specifically cocaine or methamphetamine, might disrupt mucous membranes [16]. Finally, individuals with chronic substance abuse display high rates of dental abnormalities [16]. Therefore, blood or saliva on inhalation equipment together with drug-induced lesions of mucous membranes is a potential mechanism by which noninjection drug use could result in HCV infection.

Abbreviations: Ab, antibody; Avg, average; BIMC, Beth Israel Medical Center; CI, confidence interval; ELISpot, enzyme-linked immunospot; IFN-γ, interferon-γ; HCV, hepatitis C virus; IDUs, injection drug users; N/A, not applicable; NIDUs, noninjection drug users; OD, odds ratio; SD, standard deviation; WCMC, Weill Cornell Medical College.

Correspondence: Marija Zeremski, PhD, Weill Cornell Medical College, 525 E. 68th Street, Box 319, New York, NY 10065, USA.
E-mail: maz2003@med.cornell.edu

Spontaneous clearance of HCV infection is recognized in aviremic patients through the detection of HCV-specific serologic responses. However, some people may clear the infection without developing antibodies while, in others, antibodies may develop and wane over time [17,18]. HCV-specific cellular immunity has been reported in antibody-negative persons who may have had viral exposure, such as healthcare workers [19], spouses and other family members of HCV-infected individuals [20,21], and prison inmates [17]. HCV-specific cellular immune responses have also been detected in 46–72% of seronegative IDUs, suggesting their prior exposure to the virus [22–24]. These findings suggest that cellular immunity alone may be sufficient for viral eradication. Alternatively, frequent exposure to small viral quantities may lead to the development of cellular immunity without seroconversion or productive HCV infection. Consequently, antibody measurement alone does not identify all individuals previously exposed to HCV.

We evaluated HCV-specific cellular and antibody responses in 57 NIDUs who never injected drugs. Detailed information on subjects' drug use and sexual practices was collected through comprehensive questionnaires. Associations between HCV-specific immune responses and drug administration behaviours were analyzed to determine factors that increase the likelihood of HCV infection.

MATERIALS AND METHODS

Subjects' characteristics

Fifty-seven NIDUs were recruited from the Beth Israel Medical Center (BIMC) drug detoxification program between March 2009 and September 2010. Our subjects are a subset of a larger group of drug users recruited for an ongoing Risk Factors study, described previously [25]. NIDUs were defined as persons who used heroin and/or cocaine in the prior 6 months but who had never injected an illicit drug. Briefly, research staff examined the intake records in the admission wards of the detoxification program to identify patients admitted within the past 3 days. Patients that were eligible and willing to participate were enrolled in the study and paid an honorarium of $20. To minimize the likelihood of misclassification, we enrolled both persons who had injected and those who had used but not injected illicit drugs. Subjects who consented to participation in this substudy received an additional $10. All subjects were interviewed by trained personnel using a structured questionnaire that covers demographics, sexual risk behaviours, and detailed questions on both injecting and noninjecting drug use. Fifteen healthy individuals without risk factors for HCV infection and who had never used drugs, other than marijuana smoked as a cigarette, were included as negative controls. All study participants provided a 60 mL blood sample.

The study protocol was approved by the Weill Cornell Medical College (WCMC) and BIMC Institutional Review Boards and was consistent with the standards established by the Helsinki Declaration of 1975. Written informed consent was obtained from all subjects.

Samples

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) plasma tubes and transported to WCMC for the same day processing. PBMCs were isolated using Ficoll-Hypaque density gradient centrifugation within 2–6 h of specimen collection and immediately used in an interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot) assay. Plasma was cryopreserved for future testing.

Hepatitis C virus antibody and RNA testing

Hepatitis C virus antibody and RNA testing was performed using frozen plasma samples. HCV antibodies were assayed using Ortho Vitros Eci anti-HCV antibody test (Ortho-Clinical Diagnostics, Raritan, NJ, USA). The presence of HCV RNA was determined using Roche Diagnostics Cobas Taqman Real-Time PCR test with the lower detection limit of 43 IU/mL (Roche Diagnostics, Indianapolis, IA, USA).

Enzyme-linked immunospot assay

Enzyme-linked immunospot assay was performed as previously described [23]. Briefly, 428 peptides encompassing the complete HCV genotype 1a polyprotein were pooled in 21 mixtures and used as antigens. PBMCs were plated on 96-well plates (300 000 cells/well) and stimulated in triplicate with each peptide mix and the following positive controls: phorbol mystriate acetate (Sigma Chemicals, St Louis, MO, USA), tetanus toxoid (Accurate Chemicals & Scientific Corporation, Westbury, NY, USA) and Candida albicans cellular antigen (Greer, Lenoir, NC, USA). Each plate had at least 6 no-antigen control wells, and each plate with more than 10 spot-forming cells (SFC) per no-antigen control well, on average, was eliminated from the study. Responses were considered positive if, after subtraction of the background, the mean number of SFC was ≥10 and at least greater than the mean +2 standard deviations of the SFC from healthy controls for the respective peptide mix. Patients were considered positive for HCV-specific cellular responses if more than one peptide mix was positive according to the above criteria.

Statistical analysis

We used *t* test for equality of means to assess group difference among continuous variables. We used the Pearson's chi square test for assessing the independence of predictor and outcome variables when the predictors were categorical. We also conducted logistic regression analysis for maximum-likelihood estimation and to obtain odds ratios. All tests were

conducted in Stata, version 11 (College Station, TX, USA). Statistical tests were considered significant when probability of type I error was <5% ($P < 0.05$).

RESULTS

Study subjects

Fifty-seven NIDUs and 15 healthy controls, with average ages of 44 and 45 years, respectively, were enrolled in this study (Table 1). The vast majority of NIDUs were Black (63%) or Hispanic (32%) as compared to the controls, 73% of whom were White. Sixty-eight percent of NIDUs had smoked crack compared with 37% whom had sniffed or snorted cocaine. Similar results were observed among those with HCV-specific antibody or cellular responses. Among those with positive serological responses, 73% indicated that they had smoked crack and 37% had sniffed or snorted cocaine. Similar trends were observed for patients who had detectable HCV-specific cellular responses.

Hepatitis C virus prevalence

Hepatitis C virus antibodies were detected in 11 out of 57 NIDUs (19.3%). Seven of these individuals had HCV infection, as indicated by HCV RNA positivity. The remaining four seropositive NIDUs had spontaneously resolved HCV infection in the past, as there was no detectable HCV RNA in their blood.

The ELISpot assay was performed in 51 NIDUs, and we found HCV-specific cellular immune responses in 5 (9.8%). These responses were relatively weak and narrow. In 4 individuals, we detected immune responses against two to six peptide mixes with ≤23 mean SFC/well. One subject developed immune responses to two peptide mixes with 76 and 137 mean SFC/well. None of the NIDUs with detectable cellular immunity was HCV seropositive. The ELISpot assay was not available in two seropositive individuals who spontaneously cleared HCV infection. None of the healthy controls had a positive ELISpot test. Of 51 NIDUs in whom both cellular and antibody immunity was measured, 14 (27.5%) were either seropositive or had detectable HCV-specific cellular immune responses, indicating previous exposure to the virus.

Associations between demographic and behavioural factors and HCV-specific immune responses

We analyzed potential differences in demographic and behavioural characteristics between NIDUs with HCV-specific antibody or cellular immunity and those that were negative on both Ab and ELISpot tests. We also compared

Table 1 Characteristics of NIDUs and healthy controls

	Noninjection drug users (NIDUs)				
	HCV Ab+ ($n = 11$)	HCV ELISpot + ($n = 5$)	HCV Ab- ELISpot− ($n = 37$)	Total* ($n = 57$)	Healthy controls ($n = 15$)
Average age (standard deviation)	48 (7)	44 (6)	44 (8)	44 (7)	45 (SD)
Gender, *n* (%)					
Male	10 (91)	4 (80)	31 (84)	48 (84)	5 (33)
Female	1 (9)	1 (20)	6 (16)	9 (16)	10 (67)
Ethnicity, *n* (%)					
White	0	1 (20)	1 (3)	2 (4)	11 (73)
Black	5 (45)	3 (60)	26 (70)	36 (63)	2 (13)
Hispanic	6 (55)	1 (20)	9 (24)	18 (32)	
Other	0	0	1 (3)	1 (2)	2 (14)
Ever been in jail/prison	9 (82)	4 (80)	28 (76)	42 (74)	N/A
Avg. duration of drug use (SD)	26 (10)	21 (7)	20 (10)	21 (9)	N/A
Type of drugs used, *n* (%) N/A					
Heroin smoked	0	0	0	0	
Cocaine (sniffed/snorted)	4 (37)	2 (40)	14 (38)	21 (37)	
Crack (smoked)	8 (73)	4 (80)	25 (68)	39 (68)	
Speedball (smoked)	0	0	1 (3)	1 (2)	
Speedball (sniffed/snorted)	1 (9)	1 (20)	2 (5)	4 (7)	
Street methadone	0	2 (40)	5 (14)	7 (12)	

NIDUs, noninjection drug users; HCV, hepatitis C virus; Ab, antibody; ELISpot, enzyme-linked immunospot; SD, standard deviation; N/A, not applicable.

*ELISpot was not available on six NIDUs, two of which had positive HCV Ab test.

seropositive NIDUs with those that were seronegative and those that were negative on both tests and NIDUs with positive HCV-specific cellular immunity with those that had negative ELISpot or were negative on both ELISpot and Ab tests. The results of these comparisons are presented in Table 2. We did not find any associations between HCV-specific immune responses and recent noninjection drug use practices. However, subjects who were positive on either HCV Ab or ELISpot tests were more likely to report having more casual opposite sex partners in the last 6 months ($P = 0.02$), to report having casual sex partners of the opposite gender who had ever injected drugs ($P = 0.03$), to be male who had ever had sex with a male partner ($P = 0.05$), or to have bought sex ($P = 0.03$) in the past 6 months compared to subjects who did not have serologic or cellular responses. Similarly, when we compared seropositive subjects with those who are seronegative, seropositive NIDUs had significantly more casual opposite sex partners in the last 6 months ($P = 0.02$). NIDUs with a positive ELISpot reported significantly more casual opposite sex partners that have ever injected drugs compared with NIDUs with negative ELISpot ($P = 0.01$). We did not find any demographic differences between subjects with or without HCV-specific immune responses. The modest number of subjects, the multiple outcome measures (anti-HCV, ELISpot) and the inter-correlations among the sexual behaviour variables precluded meaningful multivariate analyses.

DISCUSSION

In this study, 19.3% of analyzed NIDUs were found to be HCV seropositive, while 9.8% had detectable, although weak HCV-specific cellular immune responses. Overall, 27.5% of study participants had either HCV-specific antibodies or cellular immunity, which is considerably higher compared to the general population in which HCV prevalence is estimated to be 1.7% [3]. Our findings are well within the 2–35% HCV seroprevalence range previously reported among NIDUs [11]. Surprisingly, we did not find any associations between patterns of noninjecting drug use behaviours and HCV-specific immune responses. However, we identified multiple associations between HCV-specific immunity and sexual behaviours, including having more casual opposite sex partners, having sex partners who had ever injected drugs, buying sex, and ever having male-to-male sex.

We believe our findings do not necessarily indicate that HCV exposure occurred only though sexual activities among our subjects. Our measures of both sexual and noninjecting drug use behaviours covered only 6 months prior to the interview, and most HCV exposures probably occurred years earlier. Additionally, having an IDU sexual partner suggests a very high likelihood of contact with an HCV-infected individual. NIDUs with IDU sexual partners may engage in noninjecting drug use and sexual activities simultaneously,

Table 2 Behavioural risk factors in NIDUs according to presence or absence of HCV-specific antibody and/or cellular immune responses

Factor	Ab+ or ELISpot + Avg (SD)	Ab- and ELISpot– Avg (SD)	*P*	OR (95% CI)	Ab+ Avg (SD)	Ab– Avg (SD)	*P*	OR (95% CI)	ELISpot + Avg (SD)	ELISpot– Avg (SD)	*P*	OR (95% CI)
Number of casual opposite sex partners in last 6 months	2.8 (5.1)	0.6 (1.7)	0.02	1.3 (1.0–1.8)	3.3 (5.9)	0.7 (1.9)	0.02	1.3 (1.0–1.6)	1.8 (3.0)	1.2 (3.4)	0.7	1.0 (0.8–1.3)
	n (%)	*n* (%)			*n* (%)	*n* (%)			*n* (%)	*n* (%)		
Males reporting male sex partners (ever)	4 (29)	2 (7)	0.05	5.6 (0.9–35.4)	3 (30)	3 (8)	0.07	4.9 (0.8–29.2)	1 (25)	5 (13)	0.5	2.3 (0.2–26.3)
Casual opposite sex partner ever injected drugs	3 (21)	1 (3)	0.03	9.8 (0.9–104.2)	1 (11)	3 (7)	0.6	1.8 (0.2–19.5)	2 (40)	2 (5)	0.01	14 (1.4–137.3)
Bought sex (last 6 months)	7 (44)	6 (16)	0.03	4.0 (1.1–15.0)	5 (45)	9 (20)	0.07	3.4 (0.9–13.8)	2 (40)	11 (24)	0.4	2.1 (0.3–14.4)

NIDUs, noninjection drug users; HCV, hepatitis C virus; Ab, antibody; ELISpot, enzyme-linked immunospot assay; OD, odds ratio; CI, confidence interval; Avg, average; SD, standard deviation.

which is particularly common when stimulants, such as crack cocaine, are taken [26]. In such a case, it would be difficult to ascertain which of the two behaviours transmitted HCV. The use of stimulant drugs may also prolong sexual intercourse [27], while their vasoconstricting effects may decrease recto-vaginal secretion, consequently enhancing the possibility of tissue abrasions that can lead to blood-to-blood contact and HCV transmission.

In this study, none of the subjects had both HCV-specific antibody and cellular immunity. Of 11 seropositive individuals, 7 had chronic HCV infection while 4 had spontaneously cleared the infection. In general, individuals who do not eradicate HCV have weak or undetectable HCV-specific T-cell responses, which is partially the reason for HCV persistence [28]. Consequently, our finding of negative ELISpot results in patients with chronic HCV infection is not surprising. Additionally, our previous work showed that almost half of IDUs who spontaneously cleared HCV do not have detectable T-cell responses [23]. Therefore, as we were unable to perform the ELISpot in two of our NIDU subjects with spontaneous HCV resolution, a lack of overlap between detectable cellular and serologic HCV-specific immune responses in our study population is not unexpected.

Limitations of this work include the relatively modest number of subjects, which may have impacted on our ability to detect significant associations between noninjection drug use patterns and HCV-specific immunity. Our questions covered the preceding 6 months, while HCV exposure probably happened much earlier. Consequently, meaningful associations would occur only if recent behaviours represented long-standing patterns. Lack of questions regarding tattoos, body piercings or blood transfusions, all associated with HCV acquisition, is another limitation of the study. Finally, our behavioural data were based on self-reports, and thus are subject to possible social desirability bias. In particular, some subjects might have injected drugs, but denied doing so during the interviews. However, we did find multiple relationships between HCV immunity and socially undesirable behaviours, e.g. reporting paying for sex, which suggests that social desirability did not completely obscure relationships between behaviour and HCV exposure.

In summary, we found HCV prevalence to be much higher in this cohort of NIDUs compared to that in the general population. HCV seroprevalence was not associated with recent noninjection drug use practices and was associated with recent sexual behaviours. However, given the likely overlap between sexual and drug use partners, we believe that both noninjection drug use and sexual behaviours should be considered potential routes of HCV transmission. We would note the great difficulties in trying to separate sharing of noninjecting drug use equipment from sexual intercourse as routes of HCV transmission among NIDUs. Even though HCV prevalence is clearly elevated among NIDUs, exposure to HCV is still a relatively rare event. Thus, large number of patients would need to be followed for long periods of time in order to identify specific drug use and sexual behaviours associated with HCV exposure among NIDUs. Until additional studies are conducted, we would suggest warning NIDUs that HCV might be transmitted through both sharing of drug equipment and through sexual activity.

ACKNOWLEDGEMENTS

This study was funded in part by National Institutes of Health, grant #R01 DA 003574 (DCD) and by the Greenberg Medical Research Foundation (AHT).

REFERENCES

1 Afdhal NH. The natural history of hepatitis C. *Semin Liver Dis* 2004; 24(Suppl 2): 3–8.
2 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5: 558–567.
3 Chak E, Talal AH, Sherman KE, Schiff ER, Saab S. Hepatitis C virus infection in the United States: an estimate of true prevalence. *Liver Int* 2011; 31: 1090–1101.
4 Hahn JA, Page-Shafer K, Lum PJ *et al.* Hepatitis C virus seroconversion among young injection drug users: relationships and risks. *J Infect Dis* 2002; 186: 1558–1564.
5 Thorpe LE, Ouellet LJ, Hershow R *et al.* Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *Am J Epidemiol* 2002; 155: 645–653.
6 Des Jarlais DC, Diaz T, Perlis T *et al.* Variability in the incidence of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus infection among young injecting drug users in New York City. *Am J Epidemiol* 2003; 157: 467–471.
7 Judd A, Hickman M, Jones S *et al.* Incidence of hepatitis C virus and HIV among new injecting drug users in London: prospective cohort study. *BMJ* 2005; 330: 24–25.
8 Hagan H, Pouget ER, Des Jarlais DC, Lelutiu-Weinberger C. Meta-regression of hepatitis C virus infection in relation to time since onset of illicit drug injection: the influence of time and place. *Am J Epidemiol* 2008; 168: 1099–1109.
9 Lelutiu-Weinberger C, Pouget ER, Des Jarlais DD *et al.* A meta-analysis of the hepatitis C virus distribution in diverse racial/ethnic drug injector groups. *Soc Sci Med* 2009; 68: 579–590.
10 Mehta SH, Astemborski J, Kirk GD *et al.* Changes in blood-borne infection risk among injection drug users. *J Infect Dis* 2011; 203: 587–594.
11 Scheinmann R, Hagan H, Lelutiu-Weinberger C *et al.* Non-injection drug use and Hepatitis C Virus: a systematic review. *Drug Alcohol Depend* 2007; 89: 1–12.

12 Caiaffa WT, Zocratto KF, Osimani ML *et al.* Hepatitis C virus among non-injecting cocaine users (NICUs) in South America: can injectors be a bridge? *Addiction* 2011; 106: 143–151.
13 Hermida M, Ferreiro MC, Barral S, Laredo R, Castro A, Diz Dios P. Detection of HCV RNA in saliva of patients with hepatitis C virus infection by using a highly sensitive test. *J Virol Methods* 2002; 101: 29–35.
14 Maticic M, Poljak M, Kramar B *et al.* Detection of hepatitis C virus RNA from gingival crevicular fluid and its relation to virus presence in saliva. *J Periodontol* 2001; 72: 11–16.
15 McMahon JM, Simm M, Milano D, Clatts M. Detection of hepatitis C virus in the nasal secretions of an intranasal drug-user. *Ann Clin Microbiol Antimicrob* 2004; 3: 6.
16 Villa PD. Midfacial complications of prolonged cocaine snorting. *J Can Dent Assoc* 1999; 65: 218–223.
17 Post JJ, Pan Y, Freeman AJ *et al.* Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. *J Infect Dis* 2004; 189: 1846–1855.
18 Takaki A, Wiese M, Maertens G *et al.* Cellular immune responses persist and humoral responses decrease two decades after recovery from a single-source outbreak of hepatitis C. *Nat Med* 2000; 6: 578–582.
19 Koziel MJ, Wong DK, Dudley D, Houghton M, Walker BD. Hepatitis C virus-specific cytolytic T lymphocyte and T helper cell responses in seronegative persons. *J Infect Dis* 1997; 176: 859–866.
20 Bronowicki JP, Vetter D, Uhl G *et al.* Lymphocyte reactivity to hepatitis C virus (HCV) antigens shows evidence for exposure to HCV in HCV-seronegative spouses of HCV-infected patients. *J Infect Dis* 1997; 176: 518–522.
21 Scognamiglio P, Accapezzato D, Casciaro MA *et al.* Presence of effector CD8 + T cells in hepatitis C virus-exposed healthy seronegative donors. *J Immunol* 1999; 162: 6681–6689.
22 Freeman AJ, Ffrench RA, Post JJ *et al.* Prevalence of production of virus-specific interferon-gamma among seronegative hepatitis C-resistant subjects reporting injection drug use. *J Infect Dis* 2004; 190: 1093–1097.
23 Zeremski M, Shu MA, Brown Q *et al.* Hepatitis C virus-specific T-cell immune responses in seronegative injection drug users. *J Viral Hepat* 2009; 16: 10–20.
24 Mizukoshi E, Eisenbach C, Edlin BR *et al.* Hepatitis C virus (HCV)-specific immune responses of long-term injection drug users frequently exposed to HCV. *J Infect Dis* 2008; 198: 203–212.
25 Des Jarlais DC, Arasteh K, Perlis T *et al.* Convergence of HIV seroprevalence among injecting and non-injecting drug users in New York City. *AIDS* 2007; 21: 231–235.
26 Ratner MS (Ed). Crack Pipe as Pimp: An Ethnographic Study of Sex for Crack Exchanges. Lantham, MD: Lexington Books, 1992.
27 Kall KI. Effects of amphetamine on sexual behavior of male i.v. drug users in Stockholm—a pilot study. *AIDS Educ Prev* 1992; 4: 6–17.
28 Thimme R, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; 194: 1395–406.