MAJOR ARTICLE

Injection Drug Use Facilitates Hepatitis C Virus Infection of Peripheral Blood Mononuclear Cells

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Infection of peripheral blood mononuclear cells (PBMCs) with hepatitis C virus (HCV) has been demonstrated and has been found to play a role in relapse of HCV disease and vertical transmission of HCV. Injection drug use is thought to impair function of the immune system and induce tolerance to viruses; therefore, HCV infection of PBMCs could be more likely to occur in injection drug users (IDUs) with HCV infection. Of 108 women who tested negative for human immunodeficiency virus type 1 and positive for HCV RNA, 51 had a history of injection drug use and 57 had no known risk factor for HCV infection. HCV infection was found, by nested reverse-transcription polymerase chain reaction analysis, in the PBMCs of 33 IDUs and of 13 non-IDUs ($P = .00003$). No correlation was found between infection of the PBMCs and HCV genotype or virus load. Route of transmission and viral factors, as well as immunologic dysfunction, may play a role in viral tropism.

Infection of peripheral blood mononuclear cells (PBMCs) with hepatitis C virus (HCV) has been demonstrated by several groups [1, 2]. The role of PBMC infection with HCV is controversial, but lymphocytes may act as an HCV reservoir and may play a key role in the relapse of HCV disease after the discontinuation of IFN therapy [3] or after liver transplantation [4]. We recently demonstrated that the presence of HCV RNA in maternal PBMCs is highly associated with transmission of HCV to the newborn [5]. It has also been shown that maternal injection drug abuse, independent of other risk factors, such as coinfection with HIV type 1 (HIV-1), represents a preeminent risk factor in the vertical transmission of HCV [6, 7].

The conditions that might facilitate entry of HCV into PBMCs are still unknown. It is possible that these conditions may be associated with a particular route of infection, dysfunction of the immune system, or repeated infection with different variants of HCV. All of these conditions are present in injection drug users (IDUs). Actually, modulation of immune system function by drug use, which can promote immune system tolerance to viruses and superinfection, has been described in IDUs [8, 9]. Therefore, the aim of the present study was to evaluate the prevalence of HCV infection of PBMCs in HCV-infected women with a history of injection drug use.

PATIENTS AND METHODS

All women who were included in the study had gone to different obstetric clinics in Florence, Italy, during pregnancy to undergo a routine blood examination that was part of a prospective study of mother-to-infant transmission of HCV. All women were tested either at the time of delivery or within 1 month after childbirth. Fifty-one consecutively evaluated HIV-1–negative and HCV RNA–positive women who had a history of injection drug use were studied. Fifty-seven consecutively evaluated women who were negative for HIV-1 and

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positive for HCV RNA and who had no known risk factor for HCV infection were included in the control group.

All women who had a history of injection drug use had stopped injecting drugs before pregnancy. For ethical reasons, liver biopsies were not performed in the present study. However, 8 women with a history of injection drug use and 7 women without such a history had undergone liver biopsy in previous years. Histological examination of liver biopsy specimens obtained from these 15 women had shown evidence of minimal or mild chronic hepatitis, with no difference noted between women who had a history of injection drug use and those who did not.

Four patients who were IDUs and 3 patients who were non-IDUs had been treated with IFN >2 years previously. None of them had responded to therapy.

History of injection drug use was determined through the use of standardized questionnaires and face-to-face interviews. After verbal consent was obtained from the women, information on injection drug use was also obtained through a review of records from medical and injection drug use services.

PBMCs were obtained from all mothers at the time of delivery or within 1 month after childbirth. PBMCs were isolated from 10 mL of heparinized whole blood by centrifugation over density gradient (Lymphoprep; Nycomed Pharma) and were washed 3 times in 10 mL of PBS. Aliquots of paired samples of plasma and 10^6 PBMCs were stored at −70°C until use. Total RNA was extracted from 100 μL of plasma, 500 μL of liquid from the final PBMC washing, and 10^6 unthawed PBMCs by use of resin, chloroform, and isopropanol (RNA fast II; Molecular Systems). The presence of HCV RNA was detected by reverse-transcription PCR, as described elsewhere [5]. To avoid obtaining false-positive results caused by sample contamination, we strictly followed the preventive measures of Kwok and Higuchi [10], and negative controls were included in all experiments.

Quantitative analysis of RNA was done at the same time that the PBMC investigation was performed; the Amplicor HCV Monitor (Roche Diagnostic Systems) was used. Virus genotypes were determined for all mothers by use of a line probe assay (Innogenetics).

Data were processed by use of the SPSSX statistical package (SSPS). RNA copy numbers were reported as median and range values. Differences were evaluated by use of the nonparametric Mann-Whitney *U* test. Differences in frequencies were evaluated by use of the χ^2 test or Fisher's exact probabilities.

RESULTS

Age, main virological features, and patterns of alanine aminotransferase (ALT) levels for all study subjects are shown in table 1. All women studied had uncomplicated pregnancies. All IDUs were heroin users.

No difference in virus load was found between IDUs and non-IDUs. The ALT levels observed also did not differ between the 2 groups. HCV genotype 3 was found significantly more frequently among IDUs ($P = .02$), but no difference in the distribution of other HCV genotypes was found in a comparison of the 2 groups.

HCV RNA was demonstrated in PBMCs obtained from 33 of 51 IDUs and from 13 of 57 control subjects ($P = .00003$). HCV RNA was never found in the fluid from the final PBMC washing.

No differences were found between the ALT levels (table 2) or serum virus loads (figure 1) of mothers whose lymphocytes harbored the virus and those of mothers whose lymphocytes did not harbor the virus (median virus load, 4.11×10^5 HCV RNA copies/mL [range, 0.08–20.28 HCV RNA copies/mL] vs. 4.89×10^5 HCV RNA copies/mL [range, 0.005–12.73 HCV RNA copies/mL] [$P = .62$]).

Genotype 1 was the most frequently noted genotype of cells infected with HCV. However, no difference in serum HCV genotypes was found between women whose lymphocytes were HCV-RNA positive and women whose lymphocytes were not HCV-RNA positive (table 2).

DISCUSSION

Infection of PBMCs with HCV occurs significantly more frequently in young women with a history of injection drug use

Table 1. Main virological features and alanine aminotransferase (ALT) patterns of women included in a study of injection drug use and hepatitis C virus infection.

Finding	IDUs ($n = 51$)	Non-IDUs ($n = 57$)
Age, median years (range)	26 (22–35)	29 (23–38)
ALT level[a]		
Normal	34 (66.7)	38 (66.7)
Fluctuating	14 (27.4)	17 (29.8)
Always elevated	3 (5.9)	2 (3.5)
Hepatitis C virus genotype		
1	20 (39.2)	32 (56.2)
2	4 (7.8)	9 (15.8)
3	18 (35.3)	8 (14.0)
4	9 (17.7)	8 (14.0)
Virus load, $\times 10^5$ copies/mL		
Median	4.10	4.90
Range	0.08–20.28	0.005–13.57

NOTE. Data are no. (%) of women, unless indicated otherwise. IDU, injection drug user.

[a] ALT levels were obtained from medical histories and records.

Table 2. Alanine aminotransferase (ALT) levels, distribution of hepatitis C virus (HCV) genotypes, and virus loads among women with or without infection of peripheral blood mononuclear cells (PBMCs) with HCV.

Finding	HCV infection of PBMCs (n = 46)	No HCV infection of PBMCs (n = 62)	P
ALT level			
Normal	30 (65.2)	42 (67.8)	
Fluctuating	14 (30.4)	17 (27.4)	
Always elevated	2 (4.4)	3 (4.8)	
HCV genotype			
1	23 (50.0)	29 (46.8)	.89
2	4 (8.7)	9 (14.5)	.53
3	9 (19.6)	17 (27.4)	.47
4	10 (21.7)	7 (11.3)	.34
Virus load, $\times 10^5$ copies/mL			
Median	4.11	4.89	
Range	0.08–20.28	0.005–12.73	.62

NOTE. Data are no. (%) of women, unless indicated otherwise.

than in a similar population without parenteral exposure to injection drugs. Data from the present study also confirm previous observations that HCV genotype 3a is more frequently found in IDUs [11, 12] and that PBMC infection with HCV does not correlate with virus load or HCV genotype [5, 13]. In particular, even though a higher percentage of genotype 1 was found in infected cells, the rate reflects the distribution of HCV genotypes in a population of Italian adults with HCV infection. No difference in HCV genotypes was found between women whose PBMCs were infected with HCV and women whose lymphocytes were not infected with HCV.

Its lack of correlation with ALT levels suggests that PBMC infection with HCV probably does not correlate with disease activity. Histological examination would be helpful in clarifying this aspect, but, for ethical reason, such examination was not possible in this study. Actually, all of the women included in the study were completely asymptomatic and had normal liver function; the large majority also had ALT levels that were considered to be within the normal range.

The mechanism through which PBMC infection occurs in IDUs needs to be investigated, and different hypothesis may be suggested. Many different variants of HCV may be found in a single HCV carrier [14]. Repeated superinfection with different HCV variants probably is common in IDUs who share needles with partners who inject drugs [15]. As demonstrated elsewhere [16], different HCV variants have tropism for different cells, and it can be speculated that the presence of multiple variants makes it more likely that one of them can infect PBMCs. The fact that there remained a high prevalence of PBMC infection among women who had been IDUs but who had stopped injecting drugs at least 1 year before participating in the study is not surprising, because HCV may persist in PBMCs for years.

Virus entry into PBMCs might also be facilitated by the immune system dysfunction that is commonly found in IDUs. Actually, immunologic modification among IDUs has been recognized for several years [8, 17]. Opioids significantly inhibit production of IFN-α and IFN-β and can induce lymphocyte apoptosis. Those effects are probably mediated through the opioid receptor, because they can be reversed by the opiate receptor antagonist naloxone. Moreover, specific lymphocyte proliferative response against viral proteins is reduced by many opioids [8]. Other reports show that opiates suppress cell-mediated immunity, such as T cell–dependent antibody production by B lymphocytes or delayed-type hypersensitivity, and decrease natural killer cell activity [7]. Therefore, injection drugs, by modulating the immune response [8, 9], may facilitate immune tolerance to HCV and entry of the virus into the PBMCs.

Other hypotheses should consider the route of infection. Actually, women with no known risk of HCV infection might have been infected through the mucosal route [18], and the rate of infection of PBMCs might be different when the host is infected through the mucosal, rather than the parenteral, route. Moreover, the amount of inoculum injected is probably larger in IDUs than in patients with no known risk of infection, and the fact that a larger inoculum might facilitate infection of PBMCs cannot be excluded. This is confirmed by preliminary data obtained by our group's study of a small population of pregnant women with posttransfusion HCV infection. In that cohort, the rate of infection of PBMCs with HCV, even

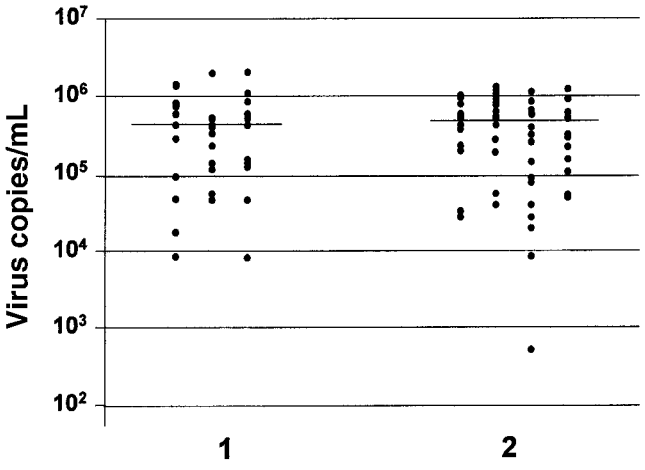

Figure 1. Serum virus loads in women with infection of peripheral blood mononuclear cells by hepatitis C virus (HCV; *1*) and in women without hepatitis C virus infection in peripheral blood mononuclear cells *(2)*. Horizontal lines denote median values.

though slightly lower than the rate demonstrated among IDUs, was higher than the rate noted among non-IDUs. The low number of patients studied prevents definitive conclusions from being drawn.

Infection of PBMCs with HCV has been reported by several groups [1], but the role of PBMC infection with HCV is controversial. It has been demonstrated that lymphocytes may act as a reservoir for HCV and may play a key role in relapse of HCV disease either after liver transplantation [4] or after the discontinuation of IFN therapy [3].

Several studies have demonstrated that injection drug use is an important risk factor for vertical transmission of HCV from HIV-positive [19] or HIV-negative [6] mothers to their infants. There is good evidence that the fetus is exposed to maternal blood and that infected PBMCs may pass from HCV-positive mothers to their offspring. Actually, maternal PBMCs may be found in cord blood samples at a frequency of 1 per 10^4–10^5 nucleated cells [20]. We recently demonstrated that maternal infection of PBMCs is crucial in mother-to-infant transmission of HCV and that the presence of HCV RNA in maternal PBMCs is highly associated with transmission of HCV to the neonate [5].

In conclusion, the results of this study show the high prevalence of PBMC infection among IDUs and contribute to explaining why maternal history of injection drug use represents an important risk factor in the vertical transmission of HCV [6, 7, 19].

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