Natural history of hepatitis C virus among injecting drug users
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Reduction of NS3 and NS5 antibodies to hepatitis C virus and increase in HCV RNA levels among HIV-coinfected injecting drug users

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Abstract
HCV infection is widely monitored by evaluating titers of antibodies to HCV proteins. A decline of titers of antibodies to HCV proteins, in particular NS3 and NS5, coincides with a decline in HCV RNA in HIV-negative individuals. Whether, these phenomena occur in the presence of HIV-coinfection is unclear. A longitudinal study of markers of HCV infection due to HCV genotype 1 was conducted in 6 HIV-coinfected individuals in comparison with 5 HIV negative individuals. In the 6 HIV-positive individuals, a quantitative loss of antibodies to all tested antigens to HCV was seen overtime, despite persistent HCV RNA levels. Comparison of this group with the HIV-negative group revealed that individuals coinfected with HIV have significant lower antibody titers to NS3 ($P=0.03$) and NS5 ($P=0.001$) while having significant higher levels of HCV RNA ($P=0.009$). With marked decline of the HCV antibodies, there was a marked decline in CD4+ cell numbers in 50% of HIV coinfected individuals. However, titers of antibodies to Core appeared most stable and persistent during HIV-coinfection. HIV infection, influences the antibody response to certain HCV antigens, leading to decreased antibody titers to NS3 and NS5 accompanied by increased HCV RNA levels. The HIV status of HCV-infected individuals must therefore be considered when NS3 and NS5 titers are used to evaluate antiviral therapy.
Introduction
Presence of antibodies to HCV can be detected by ELISA and recombinant immunoblot assays (RIBA™) and reflects chronic infection or past resolved infection with HCV. The main route of HCV transmission is parenteral and injecting drug users have a high prevalence of HCV [1]. Coinfections with other parenterally transmitted viruses are likely to occur in drug users due to needle sharing. Although, the main route of transmission of HIV is promiscuous sexual intercourse, coinfections of HCV with HIV are regularly found in injecting drug users [2].

Recently we described higher replication rates of HCV in HIV-coinfected individuals, due to an unknown direct interaction of the two viruses or to HIV-induced immunodeficiency [3]. Several reports have shown impaired HCV antibody responses and seroreversions among HIV-seropositive individuals [4,5]. In HIV-negative subjects, a decline in antibody titters to NS3 and NS5 coincides with a decline in HCV RNA (Beld et al., submitted) [6-8]. To investigate whether the same phenomenon occurs among HIV-coinfected individuals, we conducted a longitudinal study of markers of HCV infection, comparing 6 HIV-positive individuals with 5 HIV-negative individuals, all infected with HCV genotype 1.

Materials and methods

Participants
The injecting drug users were recruited from a cohort started in December 1985 [9]. Nineteen HCV seroconverters were identified [10]. Of these seroconverters, 2 were HIV seropositive at entry of the study period, whereas 4 seroconverted for HIV early during follow-up. Therefore 6 HCV seroconverting injecting drug users, persistently infected with HCV genotype 1, could be studied as to their antibody profiles in serial samples during their period of coinfection with HIV. They were compared with 5 individuals, persistently infected with HCV genotype 1, who remained HIV-negative during the study period as described earlier (Beld et al., submitted).

Serum and plasma samples were stored initially at +4°C, then frozen at -20°C within 24 hours of collection and handling, and ultimately stored at -70°C. None of the subjects received any antiviral therapy during follow-up.

Laboratory tests
The data presented here concern the following assays: Sera were tested for the presence of antibodies to HCV (EIA 3.0; Abbott Laboratories, Chicago, ILL), antibodies to HIV-1 (EIA; Abbott Laboratories, Chicago, ILL) and confirmed by Western blot analysis (Diagnostic Biotechnology, Herent, Belgium). Quantification of antibodies to Core, NS3, NS4, and NS5 proteins of HCV was performed using the Chiron RIBA™ HCV-titering Strip Immunoblot Assay (SIA) expressed as relative intensity using the automated RIBA™ Processor System (Chiron Corp., Emeryville, CA). HCV RNA was detected by RT-PCR [10] and quantified by bDNA (Chiron Corp., Emeryville, CA). The genotypes were determined either by the HCV LiPa protocol (Line Probe Assay, LiPa, Innogenetics, Ghent, Belgium) [11] or by direct-sequencing the products obtained by nested PCR of the 5'-UTR [10]. T lymphocyte immunophenotyping for CD4 membrane markers was performed on a Coulter Epics-C cytofluorometer (Coulter Electronics, Hialeah, FL). PBMC were stained with CD4-mAb (Leu-3a-PE; Becton Dickinson, Mountain View, CA). The Mann-Whitney test was used for comparison of mean values of all four quantified antibodies between groups. A value of $P < 0.05$ was considered significant.
Results

HCV antibody profiles in HIV-seropositive individuals
The 6 HIV-coinfected individuals in this study were all infected with HCV genotype 1. Elisa results remained positive during follow-up, but a gradual loss of antibodies to different antigens of HCV genotype 1 was found in all 6 individuals over time (Fig. 1).

Figure 1. Patterns of HCV viraemia and serological responses in 6 HIV-coinfected individuals. HCV viraemia was determined by RT-PCR and bDNA. PCR results are shown as plus or minus, whereas bDNA values are indicated by the bold line, expressed as HCV RNA copies/ml. The dotted line represents the detection limit of the bDNA assay. The open bar indicates EIA 3.0 results, and quantitative antibody levels to Core (circle), NS3 (star), NS4 (diamond), and NS5 (triangle) are indicated.
Antibodies to HCV genotype 1 in persons with and without HIV-coinfection

Samples with known HCV load, known HCV genotype and known antibody responses to Core, NS3, NS4, and NS5, were analyzed and used to compare individuals coinfected with HIV (n=6) versus individuals infected with HCV alone (n=5). Individuals without HIV-coinfection, had significantly higher median NS3 antibody titers (2.61 RI vs 1.40 RI; P=0.03) and median NS5 antibody titers (2.05 RI vs 0.04 RI; P=0.001) than the group of HIV-coinfected individuals, whereas Core and NS4 were not significantly different between the two groups (Fig. 2). As previously reported [3], median HCV loads in HIV co-infected individuals were significantly higher as compared to HIV negative individuals (1.0 x 10^7 HCV RNA/ml vs 2.7 x 10^6 HCV RNA/ml; P=0.009; Fig. 2).

Figure 2. Scattergram of HCV RNA load and antibody responses to Core, NS3, NS4, and NS5 in HIV-positive individuals infected with HCV genotype 1 compared to HIV-negative individuals infected with HCV genotype 1. Median values are indicated by short horizontal bars, and the detection limit of the bDNA (2 x 10^5 HCV RNA geq/ml) is indicated by the dotted line.
**CD4+ cell number in relation to HCV antibodies**

Of the 6 coinfected subjects, 3 (50%) had a significant decline in antibody responses to at least 2 antigens at the end of the study period, as well as a significant decline in CD4+ cell numbers ($P<0.05$). In contrast, individuals 0245 and 1083 had less than 500 CD4+ cell numbers/µl at entry of the study period and remained anti-Core positive until the end of follow-up. Individual 1217 had CD4+ cell numbers above 500 cells/µl during the whole study period, without a significant decline, and only the antibodies to NS4 decreased significantly ($P<0.05$). Among the 5 subjects without HIV-coinfection, none had a significant decline in antibody responses to at least 2 antigens at the end of the study period, as well as no significant decline in CD4+ cell numbers was observed (Table 1).

**Table 1. Antibody levels to 4 HCV antigens in relation to CD4+ cell numbers.**

<table>
<thead>
<tr>
<th>IDU</th>
<th>follow-up</th>
<th>CD4+</th>
<th>CD4+</th>
<th>C1</th>
<th>C2</th>
<th>NS3</th>
<th>NS3</th>
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<td>0.00</td>
<td>0.01</td>
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</table>

Note: Follow-up is in years after HIV coinfection.

1Beginning of study period during coinfection.

2End of study period.

**Discussion**

Coinfections with HCV and HIV have been studied extensively, with regard to the direct or immune-mediated influence of HIV on the replication rate of HCV [3] and on the loss of antibodies to HCV [2]. Loss of antibodies to HCV, or seroreversion, results mainly from immunological disorders caused by therapy or HIV-coinfections, but they also occur spontaneously [12]. Currently, the gold standard for the detection of HCV infection is the detection of antibodies to HCV by second or third-generation ELISA, with confirmation by RIBA™ to at least 2 antigens. We showed previously that a decline in NS3 and NS5 antibodies to HCV among HIV-negative persons was accompanied by apparent viral clearance from blood, whereas persistent viraemia was accompanied by significantly higher HCV antibody responses to NS3 and NS5, a phenomenon that may reflect the increase in antigenic mass in the presence of an intact immune system (Beld et al. Hepatology in press).

The present study shows that in HIV-positive injecting drug users a gradual loss of antibodies to HCV occurs, despite persistent HCV infection. Antibodies to HCV are more accurately detected in HIV-positive individuals by EIA 3.0 than by RIBA™, as indicated by our finding that EIA 3.0 results remained positive throughout the study period regardless of immune status, as well as an earlier finding among HCV-positive and HIV-coinfected haemophiliacs [4]. Our comparison of individuals persistently infected with HCV genotype 1 with and without HIV infection, revealed that antibody titers to NS3 and NS5 were lower in those with HIV-coinfection. Moreover, HCV RNA levels were significantly higher in HIV-coinfected in-
individuals than in individuals persistently infected with HCV who remained HIV-negative during the study period. There appeared to be no distinguishing differences in the sequence of loss of HCV antibodies, which were decaying with dissimilar rates. Antibodies to Core appeared to be most persistent, followed by antibodies to NS3, but both declined over time in individuals in whom Core and NS3 were initially detectable. Even with marked declines in CD4+ cell numbers over time, or initially low CD4+ cell numbers, antibody responses to Core were more stable, followed by NS3, NS4, and NS5. Antibodies to Core thus appear to be the most persistent and least dependent on the immune status, a finding that may be of diagnostic significance of HCV infection among patients coinfected with HIV.

These findings suggest that HIV infection influences antibody responses to certain HCV antigens and leads to significantly decreased antibody titers to NS3 and NS5 accompanied by significantly increased HCV RNA levels. These results, contrast previous findings (Beld et al., submitted) [6-8], in which HIV-negative individuals with transient HCV RNA levels or clearance of HCV RNA from blood showed significantly decreased antibody levels to NS3 and NS5. Therefore, using a decline in antibody titers to NS3 and NS5 for efficacy for monitoring HCV therapy among HIV-coinfected individuals is dissuaded.

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