Published in final edited form as:

Hepatology. 2013 June ; 57(6): 2124–2134. doi:10.1002/hep.26263.

Plasma interferon-gamma-inducible protein-10 (IP-10) levels during acute hepatitis C virus infection

Jason Grebely^{1,*}, Jordan J. Feld^{2,*}, Tanya Applegate¹, Gail V. Matthews^{1,3}, Margaret Hellard^{4,5}, Alana Sherker², Kathy Petoumenos¹, Geng Zang⁶, Ineke Shaw¹, Barbara Yeung¹, Jacob George⁷, Suzy Teutsch⁸, John M. Kaldor¹, Vera Cherepanov², Julie Bruneau⁶, Naglaa H. Shoukry⁶, Andrew R. Lloyd⁸, and Gregory J. Dore¹

Jason Grebely: jgrebely@kirby.unsw.edu.au; Jordan J. Feld: jordan.feld@uhn.ca; Tanya Applegate: tapplegate@kirby.unsw.edu.au; Gail V. Matthews: gmatthews@kirby.unsw.edu.au; Margaret Hellard: hellard@burnet.edu.au; Alana Sherker: 7as1@queensu.ca; Kathy Petoumenos: kpetoumenos@kirby.unsw.edu.au; Geng Zang: geng.zang@umontreal.ca; Ineke Shaw: ishaw@kirby.unsw.edu.au; Barbara Yeung: byeung@kirby.unsw.edu.au; Jacob George: jacob.george@sydney.edu.au; Suzy Teutsch: s.teutsch@unsw.edu.au; John M. Kaldor: jkaldor@kirby.unsw.edu.au; Vera Cherepanov: vera.cherpanov@utoronto.ca; Julie Bruneau: julie.bruneau@umontreal.ca; Naglaa H. Shoukry: naglaa.shoukry@umontreal.ca; Andrew R. Lloyd: a.lloyd@unsw.edu.au; Gregory J. Dore: gdore@kirby.unsw.edu.au

¹The Kirby Institute for Infection and Immunity in Society, University of New South Wales, Sydney, Australia

²University of Toronto, Toronto, Canada

³HIV/Immunology/Infectious Diseases Clinical Services Unit, St Vincent's Hospital, Sydney, Australia

⁴Burnet Institute, Melbourne, Australia

⁵Infectious Diseases Unit, The Alfred Hospital, Melbourne

⁶Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, Canada

⁷Storr Liver Unit, Westmead Millennium Institute and Westmead Hospital, University of Sydney, Sydney

⁸Inflammation and Infection Research Centre, School of Medical Sciences, University of New South Wales, Sydney, Australia

Abstract

Systemic levels of interferon-gamma-inducible protein-10 (IP-10) are predictive of treatment-induced clearance in chronic HCV. In the present study, factors associated with plasma IP-10 levels at the time of acute HCV detection and the association between IP-10 levels and spontaneous clearance were assessed in three cohorts of acute HCV infection. Among 300

Corresponding Author: Jason Grebely, PhD, Senior Lecturer, Viral Hepatitis Clinical Research Program, The Kirby Institute for Infection and Immunity in Society, University of New South Wales, Phone: +61-2-9385 0957, Fax: +61-2-9385 0876, jgrebely@kirby.unsw.edu.au.

*joint first authors

Disclosures: JG is a consultant/advisor for Merck. GD is a consultant/advisor and has received research grants from Roche, Merck, Janssen, Gilead, Bristol Myers Squibb.

Author Contributions: Authors GJD, GVM, MH and JMK designed the original ATACH study and wrote the protocol. Authors JGr, JJF, TA, GVM, GJD, JB, NHS and ARL designed the IP-10 sub-study. Authors JGr, JJF and GJD drafted the primary statistical analysis plan, which was reviewed by GVM, ARL, JB and NHS. TA, JGe and IS coordinated IP-10 testing and IL28B genetic sequencing. JJF, AS and VC conducted the laboratory work related to IP-10. The primary statistical analysis was conducted by JGr, JJF, GJD, JB and ARL. All authors reviewed data analysis. Author JGr wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

individuals, 245 (181 male, 47 HIV+) were HCV RNA+ at acute HCV detection. In adjusted analysis, factors independently associated with IP-10 levels ≥ 150 pg/mL (median level) included HCV RNA levels >6 log IU/mL, HIV co-infection and non-Aboriginal ethnicity. Among 245 HCV RNA+ at acute HCV detection, 214 were untreated (n=137) or had persistent infection (infection duration ≥ 26 weeks) at treatment initiation (n=77). Spontaneous clearance occurred in 14% (29 of 214). Individuals without spontaneous clearance had significantly higher mean plasma IP-10 levels at the time of acute HCV detection than those with clearance (248 ± 32 vs. 142 ± 22 pg/mL, $P=0.008$). The proportion of individuals with spontaneous clearance was 0% (0 of 22, $P=0.048$) and 16% (27 of 165) and in those with and without plasma IP-10 levels ≥ 380 pg/mL. In adjusted analyses, favourable IL28B genotype was associated with spontaneous clearance, while higher HCV RNA level was independently associated with lower odds of spontaneous clearance.

Conclusion—High IP-10 levels at acute HCV detection were associated with failure to spontaneously clear HCV. Patients with acute HCV and high baseline IP-10 levels, particularly >380 pg/mL, should be considered for early therapeutic intervention, and those with low levels should defer therapy for potential spontaneous clearance.

Keywords

IP-10; CXCL10; spontaneous clearance; acute; HCV RNA; HIV

Spontaneous clearance of hepatitis C virus (HCV) occurs in 25% of individuals (1). Female gender (1, 2), initial cellular immune response (3) and host genetics (4, 5) are associated with clearance in prospective studies of acute HCV. Polymorphisms near the *interleukin-28B* (*IL28B*) or *interferon lambda 3* (*IFN- λ 3*) gene are strongly associated with spontaneous clearance (4, 5).

Treatment responses during acute HCV are high (6), but treatment is costly and may lead to adverse events. As such, the benefits of early treatment must be balanced against the potential for spontaneous clearance. Identifying factors predicting spontaneous clearance is important for enhancing clinical decision-making around early therapeutic intervention and may also provide insight into the mechanisms involved in spontaneous clearance.

During treatment for chronic HCV, the expression level of interferon-stimulated genes (ISGs) in the liver is associated with the probability of achieving a sustained virological response (SVR) (7–11). Patients with high baseline hepatic ISG expression have a lower chance of SVR with interferon-based therapy. However, repeated liver biopsies are invasive and impractical, so serum biomarkers have been investigated. IFN- γ -inducible protein-10 (IP-10, CXCL10) is a chemokine produced by a variety of cells, including hepatocytes, attracting T lymphocytes, natural killer cells and monocytes (12). IP-10 is interferon-inducible and is produced by hepatocytes upon HCV infection (13) with circulating plasma IP-10 levels correlating with intrahepatic IP-10 mRNA expression (14) in chronic HCV infection. Similar to hepatic ISG expression, circulating IP-10 levels are predictive of treatment outcome. High pre-treatment IP-10 levels are associated with reduced rates of SVR during PEG-IFN/RBV treatment of chronic HCV (14–19) and HCV/HIV co-infection (20, 21). Further, when pre-treatment IP-10 levels are combined with *IL28B* genotype, the predictive value for discrimination between SVR and non-response is improved, especially in those with unfavourable *IL28B* genotypes (17, 18). However, there are limited data on factors associated with high levels of IP-10 and the impact of IP-10 levels on spontaneous clearance.

In this study, factors associated with IP-10 levels at the time of acute HCV detection were investigated. Additionally, we sought to evaluate the utility of plasma IP-10 levels at the time of acute HCV detection as a predictor of spontaneous clearance.

METHODS

Study design

Data from three cohorts studying acute HCV were used for this study. The Australian Trial in Acute Hepatitis C (ATAHC) was a prospective study of recent HCV (6). The Hepatitis C Incidence and Transmission Study in prison (HITS-p) is an ongoing study of prison inmates at-risk of acute HCV in correctional centers (22). The St. Luc Cohort, HEPACO study is a community-based study of people who inject drugs at risk of acute HCV (23). Follow-up is at least every six months in all cohorts.

For inclusion, participants from these cohorts had to have acute HCV defined by an initial positive anti-HCV test and either 1) a negative anti-HCV test within two years prior to the initial positive anti-HCV test or 2) acute clinical hepatitis (either jaundice or ALT >400 IU/mL) within 12 months of the initial positive anti-HCV result. Among individuals HCV antibody negative and HCV RNA positive at the time of acute HCV detection, the estimated date of HCV infection was four weeks prior to diagnosis date (4-6). Among individuals with HCV seroconversion and no acute symptomatic infection, the estimated date of infection was calculated as the mid-point between the last negative HCV antibody and first positive HCV antibody or RNA test. Among individuals with acute symptomatic infection, the estimated date of infection was calculated as six weeks prior to the onset of acute clinical hepatitis. All participants provided written informed consent and protocols were approved by local ethics committees.

Detection and quantification of HCV RNA

Qualitative HCV RNA testing was performed using the Versant TMA assay [Bayer, Australia;<10 IU/ml;ATAHC], COBAS AmpliPrep/COBAS TaqMan HCV assay (Roche, Branchburg, NJ, USA;<15 IU/ml;HITS-p) or COBAS AMPLICOR HCV Test v2.0 (Roche Diagnostics, Mannheim, Germany; <50 IU/ml;HEPCO). Quantitative HCV RNA testing was performed using the Versant HCV RNA 3.0 (Bayer, Australia;<615 IU/ml;ATAHC) or COBAS AmpliPrep/COBAS TaqMan HCV assay (Roche, Branchburg, NJ, USA;<15 IU/ml;HITS-p). HCV genotype (Versant LiPa1 or LiPa2, Bayer, Australia) was performed on all participants with detectable HCV RNA at acute HCV detection.

Laboratory testing

Participants with available plasma samples at the time of acute HCV detection were identified and plasma IP-10 was measured by an in-house enzyme-linked immunosorbent assay (ELISA, tested in duplicate) (15). IP-10 was also measured among untreated participants with available follow-up samples. *IL28B* genotype was determined by sequencing of the rs8099917 and rs12979860 single nucleotide polymorphisms (SNP) (5).

Study outcomes

In this analysis, factors associated with plasma IP-10 levels at the time of acute HCV detection were investigated. IP-10 levels were stratified by the median (150 pg/mL), consistent with the IP-10 cut-off used in previous studies (15, 16, 18, 20).

The association between IP-10 at the time of acute HCV detection and spontaneous clearance was investigated. Participants with spontaneous clearance were identified (two undetectable HCV RNA tests<10 IU/mL, 4 weeks apart) and IP-10 levels at the time of

acute HCV detection were compared to participants without clearance (untreated participants and treated participants with an estimated duration of infection of ≥ 26 weeks). Treated individuals with an estimated duration of infection < 26 weeks were excluded from analyses of spontaneous clearance to reduce misclassification bias because there is uncertainty around whether these individuals would have demonstrated spontaneous clearance in the absence of treatment. Given that IP-10 levels are lower in HCV RNA negative individuals, participants with spontaneous clearance prior to the time of detection of acute HCV (HCV RNA negative) were excluded from further analysis. Two cut-offs for plasma IP-10 levels were assessed in relation to spontaneous clearance: i) IP-10 level above and below the median (150 pg/mL) and ii) IP-10 level determined by conducting a receiver operator characteristic (ROC) curve analysis to identify the plasma IP-10 threshold maximizing the sensitivity and specificity of the association between plasma IP-10 and spontaneous clearance.

The estimated date of clearance was defined as the midpoint between the first of two consecutive undetectable qualitative HCV RNA samples and either the last sample with detectable HCV RNA or the estimated date of infection, in the event that the sample collected at the time of acute HCV detection was HCV RNA undetectable.

Statistical analyses

Baseline characteristics were compared between groups using the Student's t-test and Fisher exact test (or χ^2 test), as appropriate. Median and mean plasma IP-10 levels were assessed and stratified by characteristics hypothesized to be associated with plasma IP-10 (15–18, 24–27), including sex, age, Aboriginal ethnicity, *IL28B* genotype, estimated duration of HCV infection, HCV genotype, HCV RNA levels and HIV infection. The correlation between log HCV RNA and log plasma IP-10 levels at the time of acute HCV detection was also assessed. The Student T test (mean), Student T test with Welch's correction (mean in analyses with unequal variance between groups) and Wilcoxon rank sum test (median) were used to compare means and medians across strata, as appropriate.

Logistic regression analyses were used to estimate crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) to identify predictors of plasma IP-10 levels at the time of acute HCV detection (stratified by median) and spontaneous clearance, including all *a priori* characteristics described above. The estimated duration of infection was assessed both as a continuous variable and categorized by its median. HCV RNA levels were stratified by log transformed values (continuous and categorized into tertiles). All variables with $P < 0.20$ in bivariate analysis were considered in multiple logistic regression models using a backwards stepwise approach with factors sequentially eliminated according to the result of a likelihood ratio test. All final multivariate models included only factors that remained significant at the 0.05 level. For all analyses, statistically significant differences were assessed at $p < 0.05$; p-values are two-sided. All analyses were performed using Stata v12.0 (College Station, TX, United States).

RESULTS

Participant characteristics

A total of 299 participants were included (Figure 1; ATAHc=163, HITS-p=93 and HEPcO=43). Among those included (n=299), 54 participants with spontaneous clearance prior to the time of detection of acute HCV were excluded (Figure 1), given lower IP-10 levels in those with undetectable HCV RNA (mean plasma IP-10 level was 169 pg/mL, median 46 pg/mL).

Participant characteristics among the 245 HCV RNA positive participants at the time of acute HCV detection are shown in Table 1. Cohort differences included a higher proportion with sexual acquisition and HIV infection in ATACHC, a higher proportion of Aboriginal ethnicity in HITS-p and a higher proportion with an estimated duration of infection <26 weeks in the HEPSCO study. The mean age was 33 years (SD, 10), 75% were male, 10% were of Aboriginal ethnicity and 19% had HIV.

Factors associated with plasma IP-10 levels at the time of acute HCV detection

Plasma IP-10 levels were available for 215 of 245 individuals who were HCV RNA positive at the time of acute HCV detection (Figure 1). Plasma IP-10 levels at the time of acute HCV detection ranged from 0 to 3,071 pg/mL [median 137 pg/mL; interquartile range (IQR): 73,264; mean 245 ± 369 pg/mL]. Log plasma IP-10 levels at the time of acute HCV detection correlated with log HCV RNA levels ($P < 0.001$, $r = 0.28$, Supplemental Figure 1). The correlation between log HCV RNA and log IP-10 at the time of acute HCV detection differed by IL28B genotype. The correlation was significant in those with the favorable CC genotype (rs12979860) but borderline in those with the CT/TT genotype (CC: $r = 0.41$, $P < 0.001$; CT/TT: $r = 0.21$, $P = 0.056$, Supplementary Figure 1). Individuals with HIV had significantly higher median (239 vs. 126 pg/mL, $P = 0.001$, Figure 2B) and mean plasma IP-10 levels (390 ± 78 pg/mL vs. 208 ± 24 pg/mL, $P = 0.004$) at the time of acute HCV detection than those with HCV alone. Median plasma IP-10 levels were not significantly different between those with unfavorable and favorable IL28B genotypes (rs8099917: GT/GG, 153 pg/mL vs. TT 141 pg/mL, $P = 0.120$; rs12979860, CT/TT, 143 pg/mL vs. TT 147 pg/mL, $P = 0.188$, Figure 2). However, mean plasma IP-10 levels were higher among those with an unfavorable IL28B genotype (rs8099917: GG/GT 350 ± 62 pg/mL vs. TT 193 ± 17 pg/mL, $P = 0.019$; rs12979860: TT/CT 294 ± 46 pg/mL vs. CC 197 ± 21 pg/mL, $P = 0.057$).

Information on ALT levels, documented HCV illness with jaundice and IP-10 were available for 113 participants from ATACHC (this information was not systematically collected from other cohorts). Among this subset ($n = 113$), both median and mean plasma IP-10 levels were higher in those with ALT >100 U/L at the time of acute HCV detection (stratified by median ALT of 100 U/L; median: 242 vs. 162 pg/mL, $P = 0.003$; mean: 383 vs. 182 pg/mL, $P = 0.010$). There was no significant difference in median and mean plasma IP-10 levels among those with and without documented HCV illness with jaundice ($n = 24$, 21%; median: 196 vs. 173 pg/mL, $P = 0.214$; mean: 378 vs. 280 pg/mL, $P = 0.210$).

Factors independently associated with plasma IP-10 levels ≥ 150 pg/mL (median) at the time of acute HCV detection were assessed (Table 2). ALT and jaundice were not included in adjusted models, given only one cohort had available data. Plasma IP-10 levels ≥ 150 pg/mL occurred more often in non-Aboriginals (51% vs. 20%, $P = 0.014$), those with HCV RNA >6 log IU/mL (76% vs. 41% in those <4 log IU/mL, $P = 0.002$) and those with HIV infection (70% vs. 42%, $P = 0.002$). No differences were observed in the proportions with plasma IP-10 level ≥ 150 pg/mL by sex, age or estimated duration of HCV infection. In adjusted logistic regression analyses (Table 2), HCV RNA >6 log IU/mL [vs. <4 log adjusted odds ratio (AOR) 6.11; 95% confidence interval (95% CI) 2.11, 17.69] and HIV infection (AOR 2.11; 95% CI: 0.96, 4.61) were independently associated with plasma IP-10 levels ≥ 150 pg/mL, while individuals of Aboriginal ethnicity were less likely to have plasma IP-10 levels ≥ 150 pg/mL at the time of acute HCV detection (AOR 0.17; 95% CI: 0.05, 0.58). No difference was observed in the frequency of IL28B rs12979860 CC genotype among Aboriginals and non-Aboriginals (39% vs. 53%, $P = 0.254$).

Longitudinal plasma IP-10 levels during untreated acute HCV infection

Plasma IP-10 levels were monitored longitudinally in 20 untreated individuals with acute HCV (8 with clearance, Figure 3 and Supplemental Figure 2). Although IP-10 levels generally mirrored HCV RNA levels, there was no clear pattern that could predict clearance or persistence.

Association between plasma IP-10 levels and spontaneous clearance

Among the 245 participants who were positive for HCV RNA at the time of acute HCV detection, 214 were either untreated (n=137) or had chronic infection (persistent HCV RNA and estimated duration of infection 26 weeks) at the time of treatment initiation (n=77) and formed the study population for assessment of spontaneous clearance (Figure 1). In this group who were HCV RNA positive at acute HCV detection (n=214), spontaneous clearance occurred in 14% (29 of 214) of individuals.

Among those with available plasma IP-10 levels at acute HCV detection (n=187), individuals who failed to clear HCV spontaneously had significantly higher mean plasma IP-10 levels at acute HCV detection than those with spontaneous viral clearance (248 ± 32 vs. 142 ± 22 pg/mL, $P=0.008$, Figure 4A), however the median plasma IP-10 levels did not differ (133 vs. 103 pg/mL, $P=0.430$). Although one individual had a very high IP-10 value (3,071 pg/mL), mean IP-10 levels remained significantly higher in those without clearance excluding this individual (230 ± 27 vs. 142 ± 21 , $P=0.010$). Receiver operator characteristic (ROC) curve analysis identified an IP-10 level of 380 pg/mL as the most useful threshold associated with spontaneous clearance. No patients with a baseline IP-10 ≥ 380 pg/mL (0 of 22) achieved spontaneous clearance, compared to 16% (27 of 165) of those with IP-10 levels < 380 pg/mL ($P=0.048$, Figure 4B). There was no significant difference in the proportion with spontaneous clearance stratified by plasma IP-10 levels above and below 150 pg/mL (15%, < 150 pg/mL, vs. 13%, ≥ 150 pg/mL, $P=0.835$).

Other factors associated with spontaneous viral clearance were also examined (Table 3). Among participants with rs8099917 genotyping (n=206), clearance was higher in those with the favorable IL28B TT genotype (19%) as compared to those with unfavorable IL28B GT/GG genotypes (6%, $P=0.013$). Similarly, among participants with rs12979860 genotyping (n=202), clearance was higher in those with the favorable IL28B CC genotype (20%) as compared to those with unfavorable IL28B CT/TT genotypes (8%; $P=0.016$). There was an association with HCV RNA level at acute HCV detection and clearance. Individuals with HCV RNA levels < 4 log IU/mL were more likely to achieve spontaneous clearance (24%) as compared to those with HCV RNA of 4–6 log IU/mL (9%; $P=0.012$) and those with HCV RNA > 6 log IU/mL (7%, $P=0.060$). There was no difference in HCV RNA level by *IL28B* genotype. Although patients with unfavorable *IL28B* genotypes tended to have higher IP-10 levels at acute HCV detection, there was no association between *IL28B* genotype and IP-10 above 380 pg/mL (47% with IP-10 > 380 pg/mL were TT at rs8099917). The combination of *IL28B* genotype and plasma IP-10 levels demonstrated spontaneous clearance as follows: low IP-10 (< 380 pg/mL) and favorable rs8099917 TT *IL28B* genotype (22%), high IP-10 and favorable rs8099917 TT *IL28B* genotype (0%), low IP-10 and unfavorable rs8099917 GT/GG *IL28B* genotype (9%) and high IP-10 and unfavorable rs8099917 GT/GG *IL28B* genotype (0%, Supplemental Figure 3).

In adjusted logistic regression analyses, *IL28B* genotype (rs8099917 TT vs. GG/GT genotype, AOR 4.22, 95% CI: 1.34, 13.28, $P=0.014$) was associated with higher odds of spontaneous clearance, while higher HCV RNA level was independently associated with lower odds of spontaneous clearance (< 4 vs. 4–6 log IU/mL, AOR 0.28, 95% CI: 0.11, 0.75 and < 4 vs. > 6 log IU/mL, AOR 0.19, 95% CI: 0.04, 0.93). Given that plasma IP-10 levels

380 pg/mL were 100% predictive of not achieving spontaneous clearance, plasma IP-10 levels could not be incorporated into adjusted models of factors associated with spontaneous clearance. As a continuous variable, IP-10 levels at acute HCV detection were not associated with spontaneous clearance (OR 0.54, 95% CI:0.16, 1.87).

DISCUSSION

Identification of factors associated with spontaneous clearance after acute HCV infection has potentially important clinical and pathophysiological significance. This study of a large sample with acute HCV showed that IP-10 levels at detection of acute infection were associated with spontaneous clearance and no patients with very high IP-10 levels (> 380 pg/mL) achieved clearance. IP-10 levels correlated with HCV RNA levels at acute HCV detection and higher HCV RNA levels (> 4 log IU/mL) predicted subsequent HCV persistence, independent of *IL28B* genotype. Based on these data, early therapy may be considered in individuals with high IP-10 (> 380 pg/mL) and higher HCV RNA levels, given the low likelihood of spontaneous clearance, regardless of *IL28B* genotype.

Patients with spontaneous clearance had lower mean, but similar median, IP-10 levels at the time of acute HCV detection than those with persistence. The majority of individuals in both groups had low IP-10 levels, with a small minority among those persistence having high IP-10 levels. This suggests that high IP-10 or its correlates are not the only factors determining outcome, as many patients failed to clear despite low IP-10 levels. However, no participants with very high IP-10 levels (> 380 pg/mL) cleared, suggesting that low IP-10 is necessary but not sufficient for spontaneous clearance. The mechanisms underlying this association are unclear and IP-10 is likely a biomarker rather than a causal driver of spontaneous clearance. These findings are consistent with a study of acute HCV infection in Austria (n=62) also demonstrating that high serum IP-10 levels were negatively associated with spontaneous clearance and increased the predictive value of *IL28B* genotyping (25).

In the current study, although a threshold of IP-10 was identified above which no one went on to achieve spontaneous clearance (> 380 pg/mL), few individuals met this criterion, somewhat limiting its clinical utility. Factors independently associated with IP-10 levels at acute HCV detection above the median for the whole study cohort (> 150 pg/mL) included higher HCV RNA levels (>6 log IU/mL), HIV co-infection and non-Aboriginal ethnicity. This is consistent with previous unadjusted analyses in chronic HCV infection demonstrating that higher HCV RNA levels (15, 16) and HIV (27) are associated with higher IP-10 levels. In acute HCV, one study of nine HCV mono-infected individuals also demonstrated a correlation between higher HCV RNA and higher plasma IP-10 levels (28). In the current study, the relationship between HCV RNA and IP-10 levels differed by *IL28B* genotype. There was a strong correlation between HCV RNA and IP-10 levels in patients with the favourable genotype, but no significant correlation was seen in those with unfavourable *IL28B* genotypes. This observation may offer some insights into the significance of IP-10 in acute HCV.

Upon HCV infection, IP-10 and other ISGs are produced by hepatocytes and many other cell types. Some ISGs, like IP-10, are produced directly by viral infection without the need for interferon production (13). What determines the level of ISG expression in response to infection is unknown but clearly relates to the *IL28B* genotype (7). In chronic HCV, those with the favourable *IL28B* genotype tend to have low levels of ISG expression allowing for strong gene induction with therapeutic interferon, ultimately leading to clearance. In contrast, those with the unfavourable *IL28B* genotypes tend to have pre-activation of ISGs with near maximal expression before treatment, resulting in no further gene induction with interferon therapy and thus non-response (7–9). If ISG induction is required for clearance,

one might have anticipated that in acute HCV infection, patients with higher ISG expression would be more likely to spontaneously clear infection. If plasma IP-10 levels are a reflection of ISG expression, the opposite pattern was seen. However, the relationship between IP-10 and HCV RNA levels may help clarify this apparent paradox. In patients with the favourable *IL28B* genotype, IP-10 levels tended to be lower but correlated well with the level of HCV RNA, suggesting that in this setting, IP-10 production, and possibly production of other ISGs, is driven by and thus proportionate to the amount of virus present. However, in patients with the unfavourable genotypes, IP-10 levels were on average higher and appeared to be entirely independent of the HCV RNA level. This might suggest that the fundamental problem in those with the unfavourable genotypes is a loss of regulation of ISG induction such that ISGs are produced independent of the stimulus and therefore lead to a less coordinated viral response. Although speculative, further research on this relationship could help clarify the elusive role of the *IL28B* genotype.

Although IP-10 is an ISG, it is also an important chemotactic factor for cells of the adaptive immune response, which are very important for spontaneous clearance. Higher levels of IP-10 might be expected to drive more immune cells to the liver and thus promote viral clearance. However, recent evidence has shown that IP-10 can undergo cleavage to form an inactive version of the protein that may act as a dominant negative by binding the CXCR3 receptor without leading to chemotaxis (19). It is possible that the higher levels of IP-10 seen in patients without spontaneous clearance are due to the cleaved inactive form of IP-10. Lastly, an additional explanation is that very high IP-10 levels may lead to chemorepulsion inhibiting migration of immune cells to the liver, leading to persistent HCV infection. Understanding the role of IP-10 cleavage in HCV and other infections is clearly a priority for future studies.

The observation that individuals of Aboriginal ethnicity had a lower proportion with high plasma IP-10 levels compared to Caucasians is unexpected and notably independent of *IL28B* genotype. Black ethnicity has been associated with higher IP-10 (17, 24) and pre-activated ISG expression profiles (10) compared to Caucasians, which has been attributed to SNPs in interferon signaling pathway genes and interferon-stimulated genes but is not completely understood (11). Further research investigating the role of Aboriginal ethnicity and IP-10 levels would be interesting to clarify whether there are pathways for differential innate immune responses that might lead to lower IP-10 production and ultimately explain the higher rates of spontaneous clearance reported in Aboriginals (29).

As previously reported in chronic HCV, associations between plasma IP-10 levels and *IL28B* genotype (18, 25) and HCV genotype (16, 26) were observed in unadjusted analyses, but these were not significant in adjusted analysis. An association was observed between higher ALT and IP-10 levels in acute HCV, which is consistent with previous data in chronic HCV (27). During chronic HCV, higher plasma and intrahepatic IP-10 levels have been associated with greater inflammation (16, 26) and fibrosis (15, 16, 24, 26, 27). In one study of individuals with chronic HCV and paired liver biopsies, serum IP-10 levels at the time of liver biopsy were predictive of the development of fibrosis 3–5 years later (24). Further research is required to understand whether higher IP-10 levels early during HCV infection are predictive of subsequent fibrosis progression.

After adjusting for *IL28B* genotype, lower HCV RNA levels (<4 log IU/mL) among those HCV RNA positive at acute HCV detection was independently associated with spontaneous clearance. This is consistent with analyses demonstrating that lower HCV RNA levels are associated with spontaneous HCV clearance (30). While it has been demonstrated in a well-characterized cohort of injecting drug users followed monthly after infection that initially high HCV-RNA level (first month of infection) is predictive of spontaneous clearance, HCV

RNA levels were lower in the period 1–3 months following infection among those with spontaneous clearance (31). In the current study, the majority of HCV RNA+ individuals with acute HCV had a duration of infection >1 month and the early peak HCV RNA was likely missed. This probably explains the heterogeneity in the results observed between studies. However, the longer estimated duration of infection among those with acute infection is consistent with individuals identified in the clinical setting. As such, low HCV RNA levels could be used to predict those with an increased likelihood of spontaneous clearance and therapy could potentially be deferred in this group.

This study has some limitations. Three cohorts of individuals with acute HCV acquired mainly through injecting drug use were combined and there were some differences between cohorts. Potential unmeasured confounding factors may have influenced the observed results of the study. Also, measurement of the cleaved and uncleaved fractions of IP-10 requires storage of plasma in specialized tubes to avoid post-collection cleavage. Unfortunately, the samples used in this study were not stored to allow for measurement of cleaved IP-10, so this could not be evaluated. Finally, although an association between IP-10 levels and clearance was identified, the mechanisms underlying this remain unclear.

In a large cohort of patients with acute HCV, high IP-10 levels at acute HCV detection were associated with reduced spontaneous clearance independent of *IL28B* genotype and therefore may serve as a useful tool to prioritize patients for early antiviral therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This study was funded by the National Institutes of Health grant RO1 DA 15999-01. The Kirby Institute for Infection and Immunity in Society is funded by the Australian Government Department of Health and Ageing. The views expressed in this publication do not necessarily represent the position of the Australian Government. The HEPSCO Cohort is supported by grants from the Canadian Institutes for Health Research (CIHR) (MOP-106468 and HEO-115696) and Fonds de recherche du Québec – Santé (FRQS) AIDS and Infectious Disease Network (Réseau SIDA-MI). Roche Pharmaceuticals supplied financial support for pegylated IFN- α -2a/ribavirin. JG is supported by a National Health and Medical Research Council Career Development Fellowship. GD and AL were supported by National Health and Medical Research Council Practitioner Research Fellowships. MH and JK were supported by National Health and Medical Research Council Research Fellowship. NHS and JB hold Chercheur Boursier salary awards from the FRQS. The Burnet Institute receives funding support from the Victorian Operational Infrastructure Support Program, Department of Health, Victoria, Australia.

List of Abbreviations

HCV	Hepatitis C virus
IL28B	interleukin-28 gene
ISGs	interferon-stimulated genes
IP-10	IFN- γ -inducible protein-10
SNPs	single nucleotide polymorphisms
ROC	Receiver Operator Characteristic

References

1. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat.* 2006; 13:34–41. [PubMed: 16364080]
2. Wang CC, Krantz E, Klarquist J, Krows M, McBride L, Scott EP, Shaw-Stiffel T, et al. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. *J Infect Dis.* 2007; 196:1474–1482. [PubMed: 18008226]
3. Diepolder HM. New insights into the immunopathogenesis of chronic hepatitis C. *Antiviral Res.* 2009; 82:103–109. [PubMed: 19428600]
4. Tillmann HL, Thompson AJ, Patel K, Wiese M, Tenckhoff H, Nischalke HD, Lokhnygina Y, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. *Gastroenterology.* 2010; 139:1586–1592. 1592, e1581. [PubMed: 20637200]
5. Grebely J, Petoumenos K, Hellard M, Matthews GV, Suppiah V, Applegate T, Yeung B, et al. Potential role for Interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. *Hepatology.* 2010
6. Dore GJ, Hellard M, Matthews GV, Grebely J, Haber PS, Petoumenos K, Yeung B, et al. Effective treatment of injecting drug users with recently acquired hepatitis C virus infection. *Gastroenterology.* 2010; 138:123–135. e121–122. [PubMed: 19782085]
7. McGilvray I, Feld JJ, Chen L, Pattullo V, Guindi M, Fischer S, Borozan I, et al. Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. *Gastroenterology.* 2012; 142:1122–1131. e1121. [PubMed: 22285807]
8. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci U S A.* 2008; 105:7034–7039. [PubMed: 18467494]
9. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology.* 2007; 46:1548–1563. [PubMed: 17929300]
10. He XS, Ji X, Hale MB, Cheung R, Ahmed A, Guo Y, Nolan GP, et al. Global transcriptional response to interferon is a determinant of HCV treatment outcome and is modified by race. *Hepatology.* 2006; 44:352–359. [PubMed: 16871572]
11. Su X, Yee LJ, Im K, Rhodes SL, Tang Y, Tong X, Howell C, et al. Association of single nucleotide polymorphisms in interferon signaling pathway genes and interferon-stimulated genes with the response to interferon therapy for chronic hepatitis C. *J Hepatol.* 2008; 49:184–191. [PubMed: 18571276]
12. Zeremski M, Petrovic LM, Talal AH. The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection. *J Viral Hepat.* 2007; 14:675–687. [PubMed: 17875002]
13. Thomas E, Gonzalez VD, Li Q, Modi AA, Chen W, Noureddin M, Rotman Y, et al. HCV infection induces a unique hepatic innate immune response associated with robust production of type III interferons. *Gastroenterology.* 2012; 142:978–988. [PubMed: 22248663]
14. Askarieh G, Alsio A, Pugnale P, Negro F, Ferrari C, Neumann AU, Pawlotsky JM, et al. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology.* 2010; 51:1523–1530. [PubMed: 20186843]
15. Lagging M, Romero AI, Westin J, Norkrans G, Dhillon AP, Pawlotsky JM, Zeuzem S, et al. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatology.* 2006; 44:1617–1625. [PubMed: 17133471]
16. Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, Neumann AU, et al. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis.* 2006; 194:895–903. [PubMed: 16960776]
17. Darling JM, Aerssens J, Fanning G, McHutchison JG, Goldstein DB, Thompson AJ, Shianna KV, et al. Quantitation of pretreatment serum interferon-gamma-inducible protein-10 improves the

- predictive value of an IL28B gene polymorphism for hepatitis C treatment response. *Hepatology*. 2011; 53:14–22. [PubMed: 21254158]
18. Lagging M, Askarieh G, Negro F, Bibert S, Soderholm J, Westin J, Lindh M, et al. Response Prediction in Chronic Hepatitis C by Assessment of IP-10 and IL28B-Related Single Nucleotide Polymorphisms. *Plos One*. 2011; 6:e17232. [PubMed: 21390311]
 19. Casrouge A, Decalf J, Ahloulay M, Lababidi C, Mansour H, Vallet-Pichard A, Mallet V, et al. Evidence for an antagonist form of the chemokine CXCL10 in patients chronically infected with HCV. *J Clin Invest*. 2011; 121:308–317. [PubMed: 21183794]
 20. Falconer K, Askarieh G, Weis N, Hellstrand K, Alaeus A, Lagging M. IP-10 predicts the first phase decline of HCV RNA and overall viral response to therapy in patients co-infected with chronic hepatitis C virus infection and HIV. *Scand J Infect Dis*. 2010; 42:896–901. [PubMed: 20608766]
 21. Zeremski M, Markatou M, Brown QB, Dorante G, Cunningham-Rundles S, Talal AH. Interferon gamma-inducible protein 10: a predictive marker of successful treatment response in hepatitis C virus/HIV-coinfected patients. *J Acquir Immune Defic Syndr*. 2007; 45:262–268. [PubMed: 17414926]
 22. Teutsch S, Luciani F, Scheuer N, McCredie L, Hosseiny P, Rawlinson W, Kaldor J, et al. Incidence of primary hepatitis C infection and risk factors for transmission in an Australian prisoner cohort. *BMC Public Health*. 2010; 10:633. [PubMed: 20964864]
 23. Bruneau J, Roy E, Arruda N, Zang G, Jutras-Aswad D. The rising prevalence of prescription opioid injection and its association with hepatitis C incidence among street-drug users. *Addiction*. 2012; 107:1318–1327. [PubMed: 22248184]
 24. Zeremski M, Dimova R, Astemborski J, Thomas DL, Talal AH. CXCL9 and CXCL10 chemokines as predictors of liver fibrosis in a cohort of primarily African-American injection drug users with chronic hepatitis C. *J Infect Dis*. 2011; 204:832–836. [PubMed: 21849280]
 25. Beinhardt S, Aberle JH, Strasser M, Dulic-Lakovic E, Maieron A, Kreil A, Rutter K, et al. Serum level of IP-10 increases predictive value of IL28B polymorphisms for spontaneous clearance of acute HCV infection. *Gastroenterology*. 2012; 142:78–85. e72. [PubMed: 22192885]
 26. Zeremski M, Petrovic LM, Chiriboga L, Brown QB, Yee HT, Kinkhabwala M, Jacobson IM, et al. Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. *Hepatology*. 2008; 48:1440–1450. [PubMed: 18798334]
 27. Roe B, Coughlan S, Hassan J, Grogan A, Farrell G, Norris S, Bergin C, et al. Elevated serum levels of interferon- gamma -inducible protein-10 in patients coinfecting with hepatitis C virus and HIV. *J Infect Dis*. 2007; 196:1053–1057. [PubMed: 17763328]
 28. Zeremski M, Hooker G, Shu MA, Winkelstein E, Brown Q, Des Jarlais DC, Tobler LH, et al. Induction of CXCR3- and CCR5-associated chemokines during acute hepatitis C virus infection. *J Hepatol*. 2011; 55:545–553. [PubMed: 21256906]
 29. Grebely J, Raffa JD, Lai C, Krajden M, Conway B, Tyndall MW. Factors associated with spontaneous clearance of hepatitis C virus among illicit drug users. *Can J Gastroenterol*. 2007; 21:447–451. [PubMed: 17637948]
 30. McGovern BH, Birch CE, Bowen MJ, Reyor LL, Nagami EH, Chung RT, Kim AY. Improving the diagnosis of acute hepatitis C virus infection with expanded viral load criteria. *Clin Infect Dis*. 2009; 49:1051–1060. [PubMed: 19725787]
 31. Liu L, Fisher BE, Thomas DL, Cox AL, Ray SC. Spontaneous clearance of primary acute hepatitis C virus infection correlated with high initial viral RNA level and rapid HVR1 evolution. *Hepatology*. 2012; 55:1684–1691. [PubMed: 22234804]

Figure 1.
Participant disposition.

Figure 2. Plasma IP-10 levels at acute HCV detection among HCV RNA positive participants with available IP-10 levels stratified by A) HCV RNA level at acute HCV detection (n=181 with available quantitative HCV RNA); B) HIV infection (n=215); C) rs8099917 IL28B genotype (n=207 with available rs8099917 genotype); and D) rs12979860 IL28B genotype (n=203 with available rs12979860 genotype). Symbols indicate individual data-points, line represents median value and p-values correspond to median (Wilcoxon rank sum test).

Figure 3.
Longitudinal measurement of HCV RNA, plasma IP-10 and ALT levels among individuals with A) Spontaneous clearance; B) Spontaneous clearance and subsequent reinfection; C) Persistent HCV infection; D) Persistent HCV infection and subsequent superinfection.

Figure 4.

Association between plasma IP-10 levels at acute HCV detection and spontaneous clearance among HCV RNA positive participants with available IP-10 levels and excluding treated individuals with an estimated duration of infection <26 weeks (n=187). A) Plasma IP-10 levels at acute HCV detection stratified by spontaneous clearance (symbols indicate individual data-points, line represents mean value and p-value corresponds to a comparison of means using Student T test with Welch's correction for unequal variance); and B) Spontaneous clearance among those with plasma IP-10 levels <150 pg/mL and ≥150 and <380 pg/mL and ≥380 pg/mL (p-value compared between groups using the Fisher exact test or χ^2 test, as appropriate); and C) Spontaneous HCV clearance stratified by IP-10 level at time of acute HCV detection and IL28B genotype (rs8099917) among HCV RNA positive participants with available IP-10 levels and excluding treated individuals with an estimated duration of infection <26 weeks (n=180).

Table 1

Characteristics of participants with recent HCV and positive HCV RNA at detection of acute HCV (n=245)

	Total (n=245)	ATAHC (n=141)	HITS-p (n=61)	HEPCO (n=43)
Female sex, n (%)	62 (25)	38 (27)	16 (26)	8 (19)
Age (yrs), mean/SD	33 (10)	36 (10)	27 (6)	34 (9)
Aboriginal ethnicity, n (%) *	24 (10)	5 (4)	18 (30)	1 (2)
Mode of HCV acquisition, n (%)				
IDU	204 (83)	101 (72)	61 (100)	42 (98)
Sexual transmission	27 (11)	27 (19)	0	0 (0)
Other	14 (6)	13 (9)	0	1 (2)
IL28B genotype				
rs8099917				
GG	7 (3)	4 (3)	1 (2)	2 (5)
GT	78 (33)	46 (34)	22 (39)	10 (24)
TT	150 (64)	86 (63)	34 (60)	30 (71)
rs12979860				
TT	20 (9)	12 (9)	4 (8)	4 (10)
CT	90 (39)	54 (39)	21 (40)	15 (36)
CC	121 (52)	71 (52)	27 (52)	23 (55)
Estimated duration of infection				
Weeks, mean/SD	23 (17)	28 (17)	21 (16)	12 (12)
<26weeks	163 (67)	80 (57)	43 (70)	40 (93)
26 weeks	82 (33)	61 (43)	18 (30)	3 (7)
HCV genotype				
1	113 (46)	76 (54)	20 (33)	17 (40)
2	9 (4)	6 (4)	1 (2)	2 (5)
3	90 (37)	56 (40)	20 (33)	14 (33)
4	2 (1)	1 (1)	0 (0)	1 (2)
6	2 (1)	0 (0)	2 (3)	0 (0)
Mixed	7 (3)	2 (1)	5 (8)	0 (0)
Unknown	22 (9)	0 (0)	13 (21)	9 (21)
HCV RNA (log IU/mL) **				
Median/IQR	4.9 (3.7–5.9)	4.9 (3.5–5.9)	4.9 (4.0–5.7)	5.3 (3.7–5.4)
<4 log	67 (32)	50 (35)	14 (23)	3 (33)
4–6 log	104 (49)	60 (43)	40 (66)	4 (44)
>6 log	40 (19)	31 (22)	7 (11)	2 (22)
HIV infection, n (%)	47 (19)	47 (33)	0 (0)	0 (0)

IQR, interquartile range

* 23 of 24 participants were of Australian Aboriginal ethnicity

** HCV RNA information was not available for 34 individuals

Table 2

Factors associated with IP-10 150 pg/mL (median) among HCV RNA positive participants with available IP-10 levels at detection of acute HCV (n=215)

	IP-10 150 pg/mL (n=103) n, %	Odds Ratio (95% CI)	P	P Overall	Adjusted OR (95% CI)	P	P Overall
Male sex (vs. female)	78 (48)	0.94 (0.50, 1.77)	0.856	-	-	-	-
Age (yrs), mean/SD	33.2 (9.7)	1.02 (0.99, 1.05)	0.252	-	-	-	-
Aboriginal ethnicity (vs. non-Aboriginal)	4 (20)	0.24 (0.08, 0.75)	0.014	-	0.17 (0.05, 0.58)	0.005	-
IL28B genotype							
rs8099917 TT (vs. GG/GT)	63 (48)	0.88 (0.50, 1.55)	0.655	-	-	-	-
rs12979860 CC (vs. CT/TT)	52 (50)	1.02 (0.59, 1.77)	0.938				
Estimated duration of infection							
Weeks, mean/SD	23.3 (16.0)	1.01 (0.99, 1.02)	0.447	-	-	-	-
26 weeks (vs. <26 weeks)	36 (53)	1.34 (0.75, 2.39)	0.316	-	-	-	-
HCV genotype ***							
1	59 (57)	1.00	-	-	-	-	-
2 or 3	36 (45)	0.61 (0.34, 1.10)	0.100	0.026	-	-	-
Other	1 (10)	0.08 (0.01, 0.68)	0.020	-	-	-	-
HCV RNA (log IU/mL) **							
Mean/per 1 log IU/mL	5.1 (1.2)	1.50 (1.17, 1.93)	0.001	-	-	-	-
<4 log	22 (41)	1.00	-	-	1.00	-	-
4-6 log	45 (48)	1.34 (0.68, 2.63)	0.402	0.007	1.75 (0.86, 3.55)	0.121	0.004
>6 log	25 (76)	4.55 (1.73, 11.91)	0.002	-	6.11 (2.11, 17.69)	0.001	-
HIV infection (vs. no HIV)	30 (70)	3.13 (1.53, 6.41)	0.002	-	2.11 (0.96, 4.61)	0.062	-

* indicates row percentage

** HCV RNA not available for 34 individuals

*** individuals with missing data were excluded from adjusted analyses

Table 3

Factors associated with spontaneous clearance at detection of acute HCV infection.

	Spontaneous clearance (n=29) n, % [*]	Odds Ratio (95% CI)	P	P Overall	Adjusted OR (95% CI)	P	P Overall
Male sex (vs. female)	19 (12)	0.67 (0.29, 1.53)	0.339	-	-	-	-
Age (yrs), mean	30	0.98 (0.93, 1.02)	0.298	-	-	-	-
Aboriginal ethnicity (vs. non-Aboriginal)	3 (13)	0.95 (0.26, 3.43)	1.000	-	-	-	-
IL28B genotype							
rs8099917 TT (vs. GG/GT)	24 (19)	3.37 (1.23, 9.24)	0.013	-	4.22 (1.34, 13.28)	0.014	-
rs12979860 CC (vs. CT/TT)	21 (20)	2.85 (1.20, 6.78)	0.016	-	-	-	-
HCV genotype							
1	13 (14)	1.00	-	-	-	-	-
2 or 3	11 (12)	0.83 (0.35, 1.95)	0.662	0.935	-	-	-
Other	1 (10)	0.67 (0.08, 5.71)	0.711	-	-	-	-
HCV RNA (log IU/mL) ^{**}							
Mean/per 1 log IU/mL	1.9	0.69 (0.49, 0.98)	0.040	-	-	-	-
<4 log	14 (24)	1.00	-	-	1.00	-	-
4-6 log	8 (9)	0.30 (0.12, 0.77)	0.012	0.018	0.28 (0.11, 0.75)	0.011	0.014
>6 log	2 (7)	0.22 (0.05, 1.06)	0.060	-	0.19 (0.04, 0.93)	0.040	-
HIV infection (vs. no HIV)	5 (15)	1.17 (0.41, 3.32)	0.770	-	-	-	-
Plasma IP-10 level 150 pg/mL (vs. <150 pg/mL)	11 (13)	0.86 (0.38, 1.97)	0.725	-	-	-	-
Plasma IP-10 level 380 pg/mL (vs. <380 pg/mL)	0 (0)	-	-	-	-	-	-

^{*} indicates row percentage

^{**} HCV RNA not available for 34 individuals

^{***} individuals with missing data were excluded from adjusted analyses