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Chronic Hepatitis C Virus Infection and the Proinflammatory Effects of Injection Drug Use

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Background. Chronic inflammation, as defined by persistent immune activation, is associated with adverse clinical outcomes. People who inject drugs (PWID) have evidence of persistent immune activation. Here, in a cohort of PWID with or without hepatitis C virus (HCV) infection, we sought to dissect out the contribution of chronic HCV infection (common in PWID) from the effects of injection drug use itself.

Methods. Four groups of study volunteers were recruited: group 1 comprised active PWID; group 2, individuals who ceased injecting drugs 1–2 months before recruitment; group 3, individuals who ceased injecting drugs 3–4 months before recruitment; and group 4, healthy volunteers. Soluble and cell-associated markers of immune activation were quantified.

Results. HCV-viremic PWID have elevated levels of immune activation when compared to healthy volunteers. Cessation of injection drug use results in a decline in immune activation in the absence of HCV viremia, while HCV-viremic individuals who previously were PWID continue to harbor elevated levels of immune activation, as defined by increased levels of soluble CD14 and tumor necrosis factor α and by the presence of $CD38^+HLA\text{-}DR^+$ $CD4^+$ and $CD8^+$ T cells.

Conclusions. Immune activation, a well-defined surrogate of poor clinical outcome that is elevated in PWID, can regress to normal levels in former injection drug users who are HCV aviremic. Therefore, enhanced harm-reduction efforts should incorporate aggressive treatment of HCV infection.

Clinical Trials Registration. NCT01831284.

Keywords. HCV infection; injection drug use; immune activation.

Immune activation, mediated by the elaboration of proinflammatory cytokines, plays a direct role in the development and progression of chronic disease states, including atherosclerosis, diabetes, and cancer, as well as manifestations of aging [1–3]. Immune activation is associated with chronic hepatitis C virus (HCV) infection, a condition common in people who inject drugs (PWID) [4]. Microbial translocation, as evidenced by increased levels of soluble CD14 (sCD14), is associated with cirrhosis and increased mortality in patients with chronic HCV infection [5]. More recently it has been observed that T cells of HCV-monoinfected and HCV-coinfected individuals exhibit shortened telomere lengths and accelerated immune senescence, a sign of chronic immune activation [6]. Clinical manifestations of immune activation during chronic HCV infection include myriad extrahepatic manifestations, such as vasculitis, arthritis, Sjorgren-like sialadenitis, and renal disease [7]. The long-term consequences of immune activation associated with HCV are less well defined.

The link between immune activation and adverse clinical outcomes has been most clearly described in individuals with chronic human immunodeficiency virus type 1 (HIV-1) infection [8]. During the Strategies for Management of Antiretroviral Therapy (SMART) trial, subjects in the intermittent therapy arm experienced a higher rate of all-cause mortality [9, 10]. Higher levels of high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), and d-dimer at study entry were associated with this observation. Furthermore, post hoc analyses have revealed a strong association between sCD14, a marker of activation of the innate immune system [11], and the risk of death [12]. Finally, in addition to identifying soluble markers of inflammation as predictive of all-cause mortality, it was recognized early in the course of the HIV-1 epidemic that expression of CD38, a marker of T-cell activation, on both $CD4^+$ and $CD8^+$ T cells was an independent risk factor for disease progression [13].

Previously, we reported on a pilot study investigating immune activation in HIV-1–infected PWID. Specifically, we measured levels of immune activation in the blood and the gastrointestinal lymphoid tissue in a cohort of HIV-1–infected PWID and compared these parameters to those in healthy

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volunteers and HIV-1–infected non-PWID [14]. We found high levels of CD38 expression on $CD8^+$ T cells and increased levels of sCD14 in the blood and gastrointestinal tissue of HIV-uninfected PWID, compared with healthy volunteers. However, our data were confounded by the high prevalence of HCV seropositivity in the PWID group. As a result, we were unable to distinguish between the effects of injection drug use and active chronic HCV infection on the immune system [15]. However, our pilot study indicated operational feasibility and allowed us to design the present study, in which we have systematically distinguished between the effects of IDU and active HCV infection. To our knowledge, this is the first study to do so.

Herein, we have examined markers of immune activation in active PWID and compared them to individuals who ceased injection behavior 1–2 months and 3–4 months before study recruitment. In addition, healthy volunteers were recruited and similarly studied. This cross-sectional approach was one way of disentangling the effects of nonsterile injection from chronic infection with HCV as we posited that the background of HCV infection would be similar in the subjects with behavioral change (ie, those who had ceased injection behavior). In doing so, we would be able to understand the relative contributions of chronic infection with HCV and active injection of heroin on immune activation as reflected by the measurement of markers of immune activation in the blood and tissue.

METHODS

Study Participants and Procedures

Our multidisciplinary cross-sectional study consisted of 4 groups. Forty-eight subjects who injected heroin at least 3 times a week were enrolled in the active injection drug use group (group 1). They were HIV-1 uninfected, had no evidence of coagulopathy or any other gastrointestinal disease that would prevent biopsy, had adequate venous access for phlebotomy from a vein on an upper extremity, had a positive screening urine test for opiates (One Step Opiate Single Drug Test, Innovacon, San Diego, California), and had recent sites of drug injection by needle on examination, as well as tracks on extremities consistent with chronic injection drug use. The patients were ruled out for acute and chronic HIV-1 infection by negative results for plasma HIV-1 RNA by polymerase chain reaction (Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test v 2.0, Branchburg, New Jersey) and rapid enzyme immunochromatographic assay for HIV-1 antibodies (OraQuick Advance, Bethlehem, Pennsylvania), respectively. Forty-eight study subjects who had ceased injection behaviors for 1–2 months (group 2) and 3–4 months (group 3) were subsequently recruited and matched to group 1 for age, sex, and race/ethnicity (Table 1). Entry criteria were identical as described for the first group; however, results of urine testing for opiates were required to be negative at screening, and subjects had no evidence of recent injection on examination. Additionally, these subjects also underwent urine testing for opiates on the day of sigmoidoscopy to confirm cessation of drug use. These individuals were also tested for HIV-1 solely with point-of-care rapid tests. Eighteen subjects from group 1 who ceased injection drug use were included in either group 2 (n = 7), group 3 (n = 2), or both (n = 9). Forty-eight healthy volunteers (group 4) were recruited from the Rockefeller University Clinical Research Support Office Recruitment Data Base. All subjects were seen at Rockefeller University Hospital and gave written informed consent. Phlebotomy was performed as previously described [16]. The trial was registered with clintrials.gov (clinical trials registration NCT01831284).

Table 1. Characteristics of the Study Groups

Characteristic	Group 1	Group 2	Group 3	Group 4
Injection drug use	Active	1–2 mo earlier	3–4 mo earlier	Never
Female sex, %	22.9	22.9	22.9	25.0
Age, y, mean ± SD	42.5 ± 7.2	43.0 ± 6.3	42.4 ± 7.1	40.3 ± 8.6
Race/ethnicity, %				
Black	14.6	14.6	14.6	58.3
Hispanic	75.0	79.2	79.2	29.2
White	10.4	4.2	6.3	10.4
Methadone use, %	36 (75.0)	35 (72.9)	30 (62.5)	0
HCV status, no. (%)				
Positive	39 (81.3)	36 (75.0)	36 (75.0)	0
Viremic	24 (50.0)	29 (60.4)	25 (52.1)	0
HCV RNA load, $\log_{10}$ copies/mL plasma, mean ± SD	5.8 ± 1.1	5.9 ± 1.0	5.8 ± 0.8	NA

Abbreviations: HCV, hepatitis C virus; NA, not applicable; SD, standard deviation.

Soluble Markers of Immune Activation

Enzyme-linked immunosorbent assay (ELISA) was used to measure plasma sCD14 levels, using the Quantikine human sCD14 detection kit (R and D Systems, Minneapolis, Minnesota); hs-CRP levels, using the Quantikine human c-reactive protein detection kit (R and D Systems, Minneapolis, Minnesota); and IL-6 levels, using the human IL-6 ELISA kit (Thermo Scientific, Pierce Biotechnology Rockford, Illinois). Remaining cytokine levels were measured using the Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, California). IL-6 levels were measured singly and by multiplex.

Cellular Markers of Immune Activation

Peripheral blood mononuclear cells (PBMCs) were analyzed immediately after isolation by flow cytometry. Antibodies directed against cell-surface markers, including CD3, CD4, CD8, CD45RA, CD38, and HLA-DR, were used, and stained cells were analyzed on an LSR Fortessa (BD Biosciences, San Jose, California), using multiparameter flow cytometry [16]. Flow cytometric data was analyzed with FlowJo software (Tree Star, Ashland, Oregon).

Statistical Analysis

Mixed-effects regression models with random intercepts to account for participation in >1 group among 27 participants (ie, repeated measures) were used to compare study groups on markers of immune activation [17, 18]. Comparisons were made conditional on HCV load, a potential confounder. Single-step adjustments for multiple testing were made to control family wise error rate, using the R multcomp package [19]. A small amount of missing data (10 of 1152 [<1%] immune activation marker results) were excluded from analysis. A priori calculations determined that power was 80% to detect a difference of 0.58 standard deviations between 2 study groups (eg, active injectors and controls). All tests of statistical significance were 2-tailed, and a P value of $< .05$ was considered significant. The R statistical computing environment was used for all analysis [20].

RESULTS

Study Participants and Patient Disposition

Three hundred fifty-four subjects entered screening, with a screen failure rate of 27%. The overall retention rate between screening and study was 86%.

Study subjects were well matched across groups 1–3 (Table 1). Group 4 subjects, although comparable in age and sex, were more likely to be African American. Study subjects were subsequently classified as HCV infected and viremic, HCV-infected and aviremic due to spontaneous control, and HCV uninfected (Table 1). As these subjects were recruited and studied prior to the availability of directly acting agents to treat HCV infection with high remission rates, all aviremic HCV-infected individuals controlled HCV spontaneously. The spontaneous control rate overall was 29.7%, consistent with previous reports [21]. Importantly, the relative numbers of HCV-viremic and aviremic subjects in each study group was comparable and lacked statistical significance (Table 1). Levels of HIV viremia were comparable in groups 1, 2, and 3 (Mean (± SD) 5.8 (± 1.1), 5.9 (± 1.0), 5.8 (± 0.8) $\log_{10}$copies/mL plasma, respectively; Figure 1, Table 1).

Active injectors reported injecting heroin approximately 3 times daily on 24 out of the previous 30 days on average. Injection of heroin and cocaine was less frequent and occurred on 7 of the previous 30 days on average, and the mean frequency of injection was twice daily.

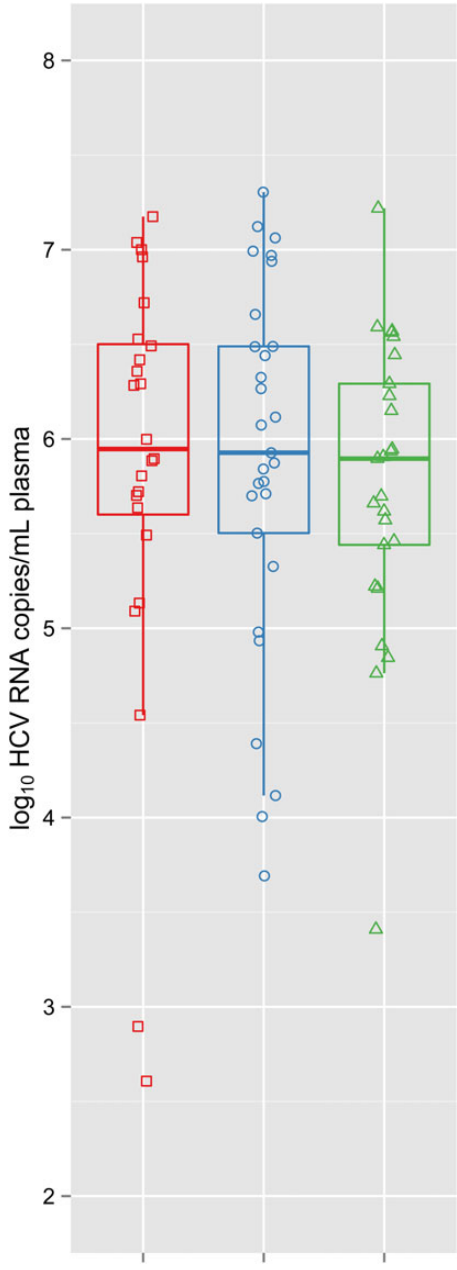

Figure 1. Levels of hepatitis C virus (HCV) RNA in study subjects. Group 1 comprised active injection drug users (squares); group 2, individuals who ceased injecting drugs 1–2 months before study recruitment (circles); and group 3, individuals who ceased injecting drugs 3–4 months before recruitment (triangles).

Soluble Markers of Immune Activation

As detailed above, we measured multiple soluble markers of immune activation either singly by ELISA or by multiplex. Formal analyses of results were performed if at least 50% of the individuals in the active injector group had levels of a particular cytokine that was above the level of detection. Cytokine levels that were at or below the level of detection in ≥50% of the active injectors included IL-6, interleukin 12p70, interleukin 15, interleukin 1β, interleukin 2, and interleukin 4.

Levels of sCD14 were measured by ELISA (Supplementary Figure 1*A*) in all subjects. We initially compared mean levels of sCD14 across groups. The mean sCD14 level was 1700 ng/mL in group 1 subjects, significantly higher than that measured in healthy volunteers (1388 ng/mL; $P < .001$), confirming our previous findings. Mean levels of sCD14 in groups 2 and 3 were 1724 and 1649 ng/mL, respectively, and not statistically different from those in active injectors or from one another. However, when we subsequently compared subgroups based

Table 2. Markers of Immune Activation, by Study Group and Hepatitis C Virus (HCV) Viremia Status

	Group 1		Group 2		Group 3		
Marker	Viremic	Aviremic	Viremic	Aviremic	Viremic	Aviremic	Group 4
sCD14 level, ng/mL	1601 ± 296	1799 ± 447	1796 ± 276	1616 ± 308	1730 ± 243	1564 ± 264	1388 ± 206
TNF-α level, pg/mL	10.7 ± 5.0	10.8 ± 7.8	12.8 ± 6.8	6.1 ± 2.9	10.6 ± 6.5	5.6 ± 2.7	6.5 ± 2.5
hs-CRP level, ng/mL	3773 ± 6677	6381 ± 5783	4133 ± 4672	3342 ± 2820	4174 ± 7262	3988 ± 5320	3413 ± 5062
$CD38^+HLA\text{-}DR^+$ $CD4^+$ T cells, %	5.3 ± 1.0	3.9 ± 1.5	4.1 ± 1.7	2.2 ± 0.6	3.9 ± 1.4	2.3 ± 0.8	2.4 ± 0.9
$CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells, %	6.4 ± 1.2	5.5 ± 1.2	5.5 ± 1.8	3.7 ± 1.1	6.5 ± 1.0	3.6 ± 1.1	4.5 ± 0.7

Data are mean ± SD. Group 1 comprised active injection drug users; group 2, individuals who ceased injecting drugs 1–2 months before study recruitment; group 3, individuals who ceased injecting drugs 3–4 months before recruitment; and group 4, individuals who never injected drugs.

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; sCD14, soluble CD14; TNF-α, tumor necrosis factor α.

on the presence or absence of HCV viremia, a different picture emerged (Table 2 and Supplementary Figure 1*B* and 1*C*). In those without measurable HCV viremia, a clear effect of ceasing injection behaviors was seen (Supplementary Figure 1*C*). Mean levels of sCD14 were 1799 ng/mL in aviremic active injection drug users, 1616 ng/mL in group 2 aviremic subjects ($P = .055$), and 1564 ng/mL in group 3 aviremic subjects ($P = .002$). Of note, mean levels of sCD14 in group 3 aviremic individuals were comparable to that measured in group 4 ($P = .106$). In contrast, in HCV viremic individuals (Supplementary Figure 1*B*) mean levels of sCD14 in groups 1, 2, and 3 were 1601, 1796, and 1730 ng/mL respectively. There was no significant difference between these levels, and all were significantly elevated when compared to those in healthy volunteers.

Levels of TNF-α were similarly measured (Table 2 and Supplementary Figure 2). The association of lower mean levels of TNF-α with the discontinuation of injection behavior was seen most clearly in the subjects who are HCV aviremic (Table 2 and Supplementary Figure 2*C*). Mean levels of TNF-α were 10.8 pg/mL in aviremic group 1 subjects, >6.1 pg/mL in aviremic group 2 subjects ($P = .002$), and 5.6 pg/mL in aviremic group 3 subjects ($P < .001$). HCV viremia masked an effect on changes in injection behavior, as mean TNF-α levels were 10.7, 12.8, and 10.6 pg/mL in the 3 subgroups respectively (Table 2 and Supplementary Figure 2*B*).

Levels of hs-CRP were highly variable and not statistically different between active injectors, healthy volunteers and groups of subjects who had ceased injection behavior (Table 2 and Supplementary Figure 3*A*). Active injectors without HCV viremia had significantly higher mean levels of hs-CRP as compared to aviremic group 2 subjects (6382 ng/mL vs 3342 ng/mL; $P = .036$; Table 2 and Supplementary Figure 3*C*); however, no statistically significant difference was seen between aviremic group 1 and aviremic group 3 subjects ($P = .16$). No differences were seen in levels of hs-CRP between groups in viremic subjects (Table 2 and Supplementary Figure 3*B*).

There were no statistically significant differences between active and former injectors when assessing levels of interferon γ, interleukin 10, and macrophage inflammatory protein 1α (data not shown).

Cellular Markers of Immune Activation

Coexpression of CD38 and HLA-DR were measured on $CD4^+$ and $CD8^+$ T cells derived from all subjects. We found that active injectors had higher mean percentages of $CD38^+HLADR^+$ cells among $CD4^+$ and $CD8^+$ T cells, compared with those measured in healthy volunteers (4.6% and 6.0% vs 2.4% and 4.5%, respectively; $P < .001$ for both comparisons; Supplementary Figures 4*A* and 5*A*).

In all patient groups, levels of activated $CD4^+$ T cells in HCV-viremic subjects were statistically significantly higher than levels in aviremic subjects (Table 2 and Supplementary Figures 4*B* and 4*C*; $P < .001$ for all comparisons). When HCV viremia was present, the mean levels of activated $CD4^+$ T cells in groups 1, 2, and 3 were not substantially different (Table 2 and Supplementary Figure 4*B*). When HCV viremia was not detectable (Table 2 and Supplementary Figure 4*C*), mean levels of activated $CD4^+$ T cells were 3.9%, 2.2% and 2.3% in groups 1, 2, and 3, respectively, with group 1 having significantly higher levels than both group 2 ($P < .001$) and group 3 ($P < .001$). Levels in groups 2 and 3 approximated that measured in group 4. Levels of activated $CD4^+$ T cells were correlated with injection frequency in those who were aviremic ($r = 0.49$, $P = .015$). This was not seen in the viremic subjects ($r = -0.08$, $P = .725$).

Mean levels of $CD38^+HLA\text{-}DR^+$ $CD8^+$ were 6.4% and 5.5% in viremic (Table 2 and Supplementary Figure 5*B*) and aviremic (Table 2 and Supplementary Figure 5*C*) active injection drug users, respectively ($P = .035$), and were also statistically significantly higher than that in healthy volunteers (4.5%; $P < .001$ for both comparisons; Table 2 and Supplementary Figure 5*A*). HCV-viremic individuals who had ceased injection behavior had markedly significantly higher mean levels of activated $CD8^+$ T cells as compared to subjects within those groups who were HCV aviremic ($P < .001$ in both comparisons); however, when compared to HCV-viremic active injectors, mean levels in HCV-viremic group 2 and 3 subjects were not

significantly different (5.5% and 6.5%, respectively). In the groups of subjects who had ceased injection behavior and were HCV aviremic (Table 2 and Supplementary Figure 5*C*), mean levels of $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells were 3.7% in group 2 and 3.6% in group 3, with both values significantly lower than that in aviremic group 1 subjects (5.5%; $P < .001$ for both comparisons). There were statistically significant lower levels of $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells in aviremic subjects in groups 2 and 3, compared with that in group 4 ($P = .013$ and $P = .001$, respectively). Levels of activated $CD8^+$ T cells did not correlate with injection frequency.

DISCUSSION

Here, we have sought to probe the intersection of injection drug use, chronic infection with HCV, and immune activation. Our previous finding of increased immune activation in blood and tissue in HIV-1–uninfected injectors in a small pilot project that led to this study was generally thought to be due in large part to the high frequency of HCV infection in the study participants, and our contention that it could be due to active injection was difficult to document [14].

Importantly, we have discovered that, in the absence of viremic HCV infection, select cell-associated and soluble markers of immune activation are higher in active injectors, compared with those who have recently ceased injection behaviors. Furthermore once HCV-aviremic individuals cease injection behaviors, select markers of immune activation approximate that measured in the healthy volunteers. Finally, HCV infection is associated with activation of the immune system and the presence of viremia masks the beneficial effects of behavioral change.

Importantly, increased levels of sCD14 [5, 12, 22] and TNF-α [23] have been associated with increased morbidity and mortality. Our observations are novel and strongly support our contention that nonsterile injection of illicit drugs, here heroin or heroin and cocaine, is associated with immune activation that, over time, may result in previously unappreciated complications of injection behavior, such as cardiovascular disease or damage to other organ systems.

Harm reduction directed at injection drug use emphasizes acute issues—infection, overdose, HIV-1 infection, and HCV infection. However, we believe we have identified potentially chronic complications of injection drug use that may be of consequence and, if shown to be the case in larger cohort studies, should perhaps be included in harm reduction messages.

With the caveat that we have only shown associations between biomarkers and behaviors, there are potential mechanisms at play, here. Active injection, being nonsterile, likely results in the injection into the circulation of immunostimulatory microbial products, which activate innate immune cells and result in the shedding and secretion of sCD14 [24, 25].

TNF-α, an acute-phase reactant, produced by macrophages and monocytes is associated with pleotropic, NF-κB–dependent immunological effects. TNF-α activates multiple cell types (such as antigen-presenting cells, endothelial cells, lymphocytes, and neutrophils) and also has potent immunosuppressive effects (such as cellular apoptosis and lymphopenia) [26]. In HCV-infected patients, TNF-α is related to higher alanine aminotransferase levels, advanced histological activity [27], and reduced viral clearance in response to interferon-based regimens [28]. Elevated TNF-α levels have been observed with injection drug use, both in HIV-1–infected and uninfected individuals [29], although the relative effects of HCV and injection drug use on TNF-α have not been studied to date.

The level of hs-CRP, yet another acute-phase reactant produced by hepatocytes in response to macrophages, is reduced in patients with HCV infection [30, 31], perhaps due to impaired hepatocyte production of hs-CRP [30]. In contrast, hs-CRP levels are elevated in PWID—users of both cocaine [32] and opioids [33]—likely reflecting injection drug use/opiate/cocaine-related immunostimulation. Accordingly, in our cohort, active injectors without HCV viremia had significantly higher mean levels of hs-CRP as compared to control subjects, while no significant differences in hs-CRP levels were observed between HCV-viremic active injectors and controls.

Here, we have also studied the expression of CD38 and HLA-DR on $CD4^+$ and $CD8^+$ T cells. CD38, an ADP ribosyl cyclase [34], is a marker of T-cell activation, with effects on cell adhesion and signal transduction. HLA-DR, the major histocompatibility complex class II cell surface receptor, is expressed on activated T cells [35] and provides evidence of T-cell stimulation. We found a striking increase in cells coexpressing CD38 and HLA-DR in both $CD4^+$ and $CD8^+$ T cells in HCV-positive active injectors, compared with normal volunteers. In the absence of HCV viremia, the levels of $CD38^+HLA\text{-}DR^+$ T cells decreased following cessation of injection drug use. In contrast, in HCV-viremic subjects, high levels of activated T cells persisted even following discontinuation of injection drug use. These data strongly suggest a role for both HCV and injection drug use in the cellular activation of lymphocytes. Interestingly, we saw a correlation between injection frequency and levels of activation in $CD4^+$ T cells in HCV-aviremic subjects, again suggesting that, when HCV is not masking the effects of injection, cellular activation is proportional to injection behavior. We hope to better understand potential mechanisms with the study of select subjects in whom longitudinal samples were collected by gene expression profiling.

Together, the present data provide a picture of heightened immune activation in PWID with concomitant HCV infection. Given our current understanding of the deleterious effects of chronic immune activation, we propose that there are likely long-term benefits resulting from aggressively treating HCV infection. A study measuring the immune effects of treating HCV with direct-acting agents is in progress to assess the immediate

immunological effects of treating HCV in both active and former injection drug users.

We believe our findings have important public health consequences. First and foremost, cohort studies probing the potentially harmful effects of immune activation caused by chronic injection drug use should be considered, particularly in HCV-aviremic and uninfected individuals. Efforts to encourage cessation of injection behaviors need to be intensified, with a new understanding of the potential long-term deleterious effects, such as immune activation. Second, individuals with chronic HCV infection should be treated aggressively with direct-acting agents, not only to treat hepatic complications of HCV, but also to prevent the complications of systemic immune activation. Such consequences, well documented in HIV-1–infected individuals, may also be true for those with chronic HCV infection. Prevention of long-term complications of immune activation may provide additional rationale in the cost-benefit analyses of these expensive medications. However, aggressive therapy of chronic HCV infection should also be accompanied by heightened harm-reduction efforts, not only to prevent reinfection but also to reduce immune activation associated with ongoing injection behaviors.

We must temper our conclusions with these caveats. Our findings are based on a relatively small cohort, given the number of viremic and aviremic individuals in each of the study groups. Our results are therefore of interest but require confirmation in larger numbers of subjects. We cannot rule out direct effects of the drugs of abuse as mediators of immune stimulation, as opposed to the nonsterile injection of heroin and cocaine. It is noteworthy that the frequency of methadone use among individuals in study groups 1, 2, and 3 was comparable; this would suggest that, at a minimum, our observations of differences between groups are unlikely to represent selective effects of opioids. Furthermore, we did not find universal elevation of levels of markers of immune activation. For example, levels of IL-6, a commonly measured cytokine that, when present at elevated levels, is associated with increased mortality and morbidity in individuals with HIV-1 infection, among other conditions, were not elevated in our study subjects. Although this and other cytokines may be produced excessively, they may be active locally in tissue and cleared from the systemic circulation rapidly, accounting for our findings. Finally, although we have documented an association between injection drug use and select markers of immune activation, we have assumed that complications of immune activation seen in other disease states may apply here.

In closing, we have documented a clear immunostimulatory effect of both injection drug use and chronic HCV infection on select immune parameters, both cell associated and soluble. Behavioral change is associated with normalization of select parameters in the absence of HCV viremia. However, behavioral change in the presence of HCV viremia is not. The clinical significance of these provocative findings requires further study in larger cohorts of relevant study subjects over the long term.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci **2014**; 69 (suppl 1):S4–9.
2. Varadhan R, Yao W, Matteini A, et al. Simple biologically informed inflammatory index of two serum cytokines predicts 10 year all-cause mortality in older adults. J Gerontol A Biol Sci Med Sci **2014**; 69:165–73.
3. Howcroft TK, Campisi J, Louis GB, et al. The role of inflammation in age-related disease. Aging **2013**; 5:84–93.
4. Li K, Lemon SM. Innate immune responses in hepatitis C virus infection. Semin Immunopathol **2013**; 35:53–72.
5. Sandler NG, Koh C, Roque A, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology **2011**; 141:1220–30, 30 e1–3.
6. Grady BP, Nanlohy NM, van Baarle D. HCV monoinfection and HIV/HCV coinfection enhance T-cell immune senescence in injecting drug users early during infection. Immun Ageing **2016**; 13:10.
7. Jacobson IM, Cacoub P, Dal Maso L, Harrison SA, Younossi ZM. Manifestations of chronic hepatitis C virus infection beyond the liver. Gastroenterol Hepatol **2010**; 8:1017–29.
8. Lichtfuss GF, Hoy J, Rajasuriar R, Kramski M, Crowe SM, Lewin SR. Biomarkers of immune dysfunction following combination antiretroviral therapy for HIV infection. Biomark Med **2011**; 5:171–86.
9. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med **2008**; 5:e203.
10. Strategies for Management of Antiretroviral Therapy Study GroupEl-Sadr WM, Lundgren J, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med **2006**; 355:2283–96.
11. Pugin J, Schurer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A **1993**; 90:2744–8.
12. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis **2011**; 203:780–90.
13. Sauce D, Elbim C, Appay V. Monitoring cellular immune markers in HIV infection: from activation to exhaustion. Curr Opin HIV AIDS **2013**; 8:125–31.
14. Mehandru S, Deren S, Kang SY, et al. Behavioural, mucosal and systemic immune parameters in HIV-infected and uninfected injection drug users. J Addict Res Ther **2015**; 6:1–8.
15. Horner SM. Activation and evasion of antiviral innate immunity by hepatitis C virus. J Mol Biol **2014**; 426:1198–209.
16. Mehandru S, Poles MA, Tenner-Racz K, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. J Exp Med **2004**; 200:761–70.

17. Bates D, Maechler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw **2015**; 67:1–48.
18. Kuznetsova A, Brockoff PB, Haubo R, Christensen B. Christensen RHB. lmerTest: tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package, version 2.0-29. **2015**. http://CRAN.R-project.org/package=lmerTest.
19. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. Biometrical J **2008**; 50:346–63.
20. Core TR. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, **2015**.
21. Wiessing L, Ferri M, Grady B, et al. Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. PloS One **2014**; 9:e103345.
22. Marchetti G, Nasta P, Bai F, et al. Circulating sCD14 is associated with virological response to pegylated-interferon-alpha/ribavirin treatment in HIV/HCV co-infected patients. PLoS One **2012**; 7:e32028.
23. Bradley JR. TNF-mediated inflammatory disease. J Pathol **2008**; 214:149–60.
24. Hiki N, Berger D, Prigl C, et al. Endotoxin binding and elimination by monocytes: secretion of soluble CD14 represents an inducible mechanism counteracting reduced expression of membrane CD14 in patients with sepsis and in a patient with paroxysmal nocturnal hemoglobinuria. Infect Immun **1998**; 66:1135–41.
25. Landmann R, Knopf HP, Link S, Sansano S, Schumann R, Zimmerli W. Human monocyte CD14 is upregulated by lipopolysaccharide. Infect Immun **1996**; 64:1762–9.
26. O'Shea JJ, Ma A, Lipsky P. Cytokines and autoimmunity. Nat Rev Immunol **2002**; 2:37–45.
27. Zylberberg H, Rimaniol AC, Pol S, et al. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. J Hepatol **1999**; 30:185–91.
28. Dill MT, Duong FH, Vogt JE, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. Gastroenterology **2011**; 140:1021–31.
29. Ownby RL, Kumar AM, Benny Fernandez J, et al. Tumor necrosis factor-alpha levels in HIV-1 seropositive injecting drug users. J Neuroimmune Pharmacol **2009**; 4:350–8.
30. Salter ML, Lau B, Mehta SH, Go VF, Leng S, Kirk GD. Correlates of elevated interleukin-6 and C-reactive protein in persons with or at high risk for HCV and HIV infections. J Acquir Immune Defic Syndr **2013**; 64:488–95.
31. Castello G, Scala S, Palmieri G, Curley SA, Izzo F. HCV-related hepatocellular carcinoma: from chronic inflammation to cancer. Clin Immunol **2010**; 134:237–50.
32. Siegel AJ, Mendelson JH, Sholar MB, et al. Effect of cocaine usage on C-reactive protein, von Willebrand factor, and fibrinogen. Am J Cardiol **2002**; 89:1133–5.
33. Reece AS. High-sensitivity CRP in opiate addiction: relative and age-dependent elevations. Cardiovasc Toxicol **2012**; 12:149–57.
34. Malavasi F, Deaglio S, Funaro A, et al. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. Physiol Rev **2008**; 88:841–86.
35. Ko HS, Fu SM, Winchester RJ, Yu DT, Kunkel HG. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells. J Exp Med **1979**; 150:246–55.