Published in final edited form as:

J Acquir Immune Defic Syndr. 2017 May 01; 75(1): e8–e12. doi:10.1097/QAI.0000000000001270.

The Relationship Between Injection Drug Use Risk Behaviors and Markers of Immune Activation

Sherry Deren, PhD¹, Charles M. Cleland, PhD¹, Haekyung Lee, PhD², Saurabh Mehandru, MD², and Martin Markowitz, MD³

¹Center for Drug Use and HIV Research, New York University Rory Meyers College of Nursing, New York, NY

²Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, New York, NY

³Aaron Diamond AIDS Research Center and The Rockefeller University, New York, NY

Abstract

High levels of immune activation are reported for people who inject drugs (PWID). Studies of the relationship between injection behaviors and immune activation have yielded mixed results, in part due to lack of control for HCV in analyses. This study, of 48 HIV-seronegative PWID, examines this relationship controlling for HCV viremia. Frequency of injection was positively related to markers of immune activation (sCD14, %CD8+CD38+HLADR+T cells), as was duration of injection (hs-CRP and D-dimer). Sharing injection equipment was not related to markers studied. Findings suggest that efforts to encourage injection cessation or reduction in frequency can have positive health benefits through reducing immune activation.

Keywords

IDU; immune activation; HIV risk behaviors

Introduction

Use of heroin and other illicit drugs has been shown to trigger an immunological response^{1,2} and high levels of immune activation have been reported for people who inject drugs (PWID).^{3,4} Increasing our understanding of the factors contributing to this heightened immune activation, which has been associated with progression of chronic disease and disability,^{5,6} can lead to better health outcomes for PWID.

Studies of the relationship between injection-related behaviors and immune function have focused primarily on people who were HIV-infected, and have not yielded consistent results. For example, while a lower rate of HIV-1 related disease progression was found among those who frequently borrowed injection equipment,⁷ others have found no relationship

between sharing injection equipment and the rate of CD4+ cell decline,⁸ and no relationship between frequency of injection and CD4+ cell decline.⁹ A review of studies on the role of substance abuse in HIV disease progression noted that the mixed results pointed to the need for further research, and that both laboratory and epidemiological studies are needed.¹ Adding further to the mixed results regarding the influence of injection behaviors on immune activation, a recent study conducted with HIV seronegative PWID reported heightened immune activation related to needle sharing.³

One contributing factor to these mixed results may be the lack of attention to Hepatitis C virus, hyperendemic worldwide among PWID¹⁰ and recently found to be related to immune activation.¹¹ This paper will examine the relationship between injection drug use behaviors, including frequency of injection and sharing injection equipment, and immune activation in a sample of HIV seronegative PWID. The analysis will control for the presence of HCV viremia.

Methods

Participants and Recruitment

As part of a study exploring the relationship between injection drug use, HCV infection and immune activation, 48 HIV seronegative PWID were recruited in NYC in 2012–14.¹¹ Recruitment occurred at needle exchange programs, other harm reduction programs, and locations where PWID might be found (e.g., near drug treatment programs, NGOs serving PWID and through NYC-based research projects). Presentations and distribution of printed recruitment materials and face-to-face interactions with project recruiters at the various venues were conducted with the assistance of referrals by program staff. Criteria for participation of PWID included: current heroin injection (at least 3 times/week), HIV-uninfected and aged 18–55.

Procedures

At the first contact, usually in a program or on the street, the project was briefly described to potential participants, including incentives and information about the procedures involved (interview, blood draws, and sigmoidoscopy). Two experienced recruiters verified current injection through examination of track marks and question probes. Those meeting the initial criteria were scheduled for an appointment at the research hospital setting where the informed consent was administered, including a detailed discussion to ensure the participant understood the project procedures before agreeing to participate. After consent was obtained, a brief computer-assisted interview was administered, collecting data on (a) sociodemographic characteristics, including age, gender, race/ethnicity, residential status; (b) injection drug use behaviors: age first injected and current injection behavior, including types of drugs, frequency, syringe sharing, injection equipment sharing (cookers, cottons, rinse water); and (c) non-injection drug use (alcohol and other substances).

After the interview was completed, a medical history and physical exam were conducted to verify eligibility for the study and identify any exclusionary medical conditions. A urine screen positive for opiates was required and HIV-seronegative status was confirmed by rapid

point-of-care serologic testing and a determination of HIV-1 RNA in plasma to rule out acute HIV-1 infection. The sigmoidoscopy was scheduled at this time, and was conducted approximately two weeks after screening. After results of phlebotomy and urine collection were reviewed, inclusion criteria (HIV seronegative, positive heroin toxicology) and exclusion criteria (e.g., severe liver disease) were assessed. Those who did not meet the criteria for the procedure were contacted and the results and the reason they did not qualify were explained and if indicated, a referral for follow up care was provided. Those who met the criteria were telephoned the night before their appointment as a reminder. At the scheduled date they came to the research hospital setting where a flexible sigmoidoscopy with biopsy was performed after phlebotomy for immunologic evaluations (see Mehandru et al.⁴ for description of procedure). Cash incentives were provided for both the screening visit and the sigmoidoscopy with biopsy. All protocols were initially reviewed and approved by the Rockefeller University IRB, with subsequent approvals obtained from the IRBs of the NYU School of Medicine and the Mount Sinai School of Medicine. This paper uses baseline data for all the active PWIDs who completed the study.

Measures

Immune activation was quantified using cell-associated markers (the expression of HLA-DR and CD38 on CD4+ and CD8+ T cells), soluble markers (soluble CD14 [sCD14] and Tissue Necrosis Factor- α [TNF- α]), and select acute phase reactants (high specificity-C reactive protein [hs-CRP] (see detail of procedures in Markowitz et al.¹¹) and D-dimer [a fibrin degradation product]) measured by enzyme immunoassay (Asserachrome D-DI). HCV viral load was determined commercially using a PCR-based assay (LabCorp).

Three types of injection risk behavior were analyzed: (a) a summary measure of sharing injection equipment (included sharing needles/syringes, backloading [i.e., having others squirt drugs from their syringes into the participant's syringe] or sharing injection paraphernalia [i.e., cookers, cotton, or rinse water]). Participants were asked the number of times they engaged in these behaviors in the prior 30 days; (b) duration of injection frequency in years; and (c) recent injection frequency, as indicated by the number of days injecting in the prior 30 days.

Data analysis

Associations between markers of immune activation and injection behaviors were estimated in linear regression models. The model for each biomarker included age (known to be related to immune activation markers),¹² years injecting, days injecting in the prior 30 days, and HCV viremia.

Injection risk sharing behaviors were considered for addition to each linear regression model if adding one or more of those variables would increase significantly the proportion of variance in the biomarker accounted for by age, years injecting, days injecting in the prior 30 days, and HCV viremia. Risk behavior variables were transformed (base-10 logarithm) before analysis to reduce positive skew in those variables.

Pearson correlations were used to estimate bivariate associations between markers of immune activation and injection behaviors separately for HCV viremic and aviremic

participants. They were also used to estimate bivariate associations between markers of immune activation and frequency and duration of injection drug use.

All analysis was conducted in the R statistical computing environment.¹³ The R effects package¹⁴ was used to plot linear model predictions of biomarkers of immune activation, along with confidence intervals.

Results

Sample Characteristics

A total of 201 potential PWID participants were scheduled for the screening appointment and half (100) kept their appointment. Close to half (43) failed screening, with primary reasons being negative toxicology (51%) (positive urines for heroin were required), and inability to draw venous blood (23%). Of those eligible for study, almost 90% (48) successfully completed the procedure and comprised the sample analyzed for this paper.

Table 1 provides sociodemographic and injection risk behavior information for the participants. Almost three-quarters (77%) were male, the mean age was 42.5, and 90% were minority (African-American and Hispanic). The majority of participants were HCV positive (81.3%), and half were viremic. Since participants for this study were recruited prior to availability of directly acting agents to treat HCV infection, aviremia among those who were HCV-infected was due to spontaneous control and not treatment.

Drug-related behaviors are summarized in Table 1. The average number of years of injection was 16.4 (sd=10.3), and number of injections in the prior 30 days, was 158.4 (sd=200.6). About 21% reported sharing needles, 4% reported backloading, 29.2% shared other paraphernalia and 35% reported any sharing behaviors.

Relationship between injection behaviors and immune activation

As noted, all analyses were conducted controlling for age, years injecting, days injecting in the past month, and HCV viremia. More days injecting in the prior 30 days was associated with higher sCD14 (B=18.4; 95% CI: 0.7 – 36.1, p=0.042) (Figure 1A) and higher levels of %CD8+CD38+HLADR+ T cells, but with only marginal significance (B=0.05, 95% CI: 0.00 – 0.11, p=0.057) (Figure 1B). More years injecting was associated with higher levels of hs-CRP (B=0.03; 95% CI: 0.01 – 0.05, p=0.008). (Figure 1C) and higher levels of D-dimer (B=0.01; 95% CI: 0.002 – 0.026, p=0.024) (Figure 1D). Bivariate, unadjusted correlational analyses were also conducted, and the results were similar to those in regression models, i.e., days injecting was related to sCD14 (r= .25, p=.08) and %CD8+CD38+HLADR+T cells (r=.31, p=.03), and years injecting was related to hs-CRP (r=.31, p=.03) and D-dimer (r=.36, p=.01).

None of the variables summarizing the number of times sharing occurred were related to any of the markers of immune activation. Correlational analyses were conducted between sharing and the immune activation markers studied, separately for HCV viremic and non-viremic participants. For those who were aviremic, there was a significant correlation between sharing and CD4+CD38+HLA DR+T (r=.44, p=.03), and no significant

associations were found for those who were viremic. In addition, none of the injection-related variables examined were related to TNF- α or to CD4+CD38+HLA DR+T cells.

In sensitivity analysis, number of days injecting in the prior 30 days was replaced with total number of times injecting in the prior 30 days. Number of times injecting was associated with higher sCD14, but with only marginal significance ($p=0.098$). Number of years injecting ($p=0.033$) and number of times injecting ($p=0.032$) were each associated with higher hs-CRP. Years injecting was associated with higher D-dimer, but with only marginal significance ($p=0.058$). Number of times injecting was not related to mean level of %CD8+CD38+HLADR+ T cells.

Discussion

Our findings indicate that duration of injection (as measured by the total number of years injected) and recent injection frequency (assessed by the number of days injected in the prior 30 days) were positively correlated with select markers of immune activation. Sharing of injection equipment (needles and paraphernalia) was not significantly related to the immune activation markers studied. While this differs from the results reported by Tomescu et al.³ this discrepancy may be attributed to the many differences between the studies. For example, Tomescu looked at NK cells, while our analysis used T cells; our analysis controlled for HCV viremia; and there were differences in the characteristics of the participants recruited (e.g., frequency of injection was lower in the Tomescu study- about once per day compared with an average of over 5 times per day in our sample). Furthermore, frequency of needle sharing appeared to be higher in the Tomescu study, however, since the time periods studied differed (6 months vs 30 days), this was not directly comparable.

The relationship between the number of injection exposures (length of time injecting and frequency) and immune activation found in the current study may be attributable to contaminants in the injection materials themselves, and more detailed study of the causal chain is needed. We have shown the relationship between HCV viremia and immune activation, and that cessation of injection among PWID who are HCV aviremic can result in normalization of select markers of immune activation.¹¹ These results together indicate that while cessation of injection has positive effects on immune activation, harm reduction efforts that reduce injection frequency (even if not leading to cessation) can also have salutary effects by reducing immune activation.

Importantly, several studies of HIV-exposed seronegative (HESN) PWID have reported higher levels of immunological activation. For example, Tran et al studied a cohort of Vietnamese HESN and observed heightened levels of immune activation parameters (as defined by CD38, HLA-DR and CD25) in HESN than in the controls, similar to the data reported in the present study.¹⁵ Recent data by Kallas et al also show a similar increase in immune activation parameters (as defined by the expression of HLA-DR and CCR5 on CD4+ and CD8+ T cell subsets) in PWID who were seronegative for HIV-1, HCV and HBV, when compared to non-IDU controls.¹⁶ Higher immune activation among other HESN has also been reported among other groups,¹⁷ including sex workers¹⁸ and infants born to HIV-infected women.¹⁹ Heightened levels of immune activation in PWID may be associated with

anti-HIV-1 activity as proposed by Tran et al.¹⁵ Alternatively, activated immune cells may enhance HIV-1 susceptibility. This supports the need for further study of the relationships between injection risk behaviors, immune activation, and HIV transmission. We are currently pursuing research strategies to better understand the complex interplay between immune activation and the risk of HIV-1 acquisition using systems-based approaches as previously reported by Burgener et al.²⁰

Another important conclusion from this study was the demonstration that active PWID can be recruited to participate in studies of immune activation, even those involving biomedical procedures such as a sigmoidoscopy.²¹ While the loss of participants from recruitment to screening was substantial, at approximately 50%, this is not surprising given the unstable life styles of many PWID. Importantly, close to 90% of those who were screened and found eligible for the study completed all components.

Further research on the relationship between injection-related behaviors and immune activation is needed. The disparate findings in studies of the relationship between injection behaviors and markers of immune activation highlight the need for undertaking studies based on larger samples, with variability in duration of injection, injection frequency and injection related sharing behaviors, to disentangle the role of specific injection-related behaviors and of other influences on immune activation. We have demonstrated the importance of considering HCV viremia in studies of immune activation among PWID.¹¹ Thus, studies also must examine the impact of HCV viremia. Our results showing a significant correlation for sharing behaviors and an immune activation marker for HCV aviremic participants indicate that separate analyses by HCV viremia status, rather than simply controlling for HCV viremia as a main effect, may be indicated. In addition to injection-related behaviors, age-related comorbidities as well as life style factors²² must be considered in studies of immune activation among PWID.

Acknowledgments

This research was supported by funds from the National Institute on Drug Abuse (R01DA033777, Markowitz; and P30DA011041, Deren and Hagan). The content is the sole responsibility of the authors and does not necessarily represent the official views of the NIH or any of the organizations involved in this research. The authors wish to acknowledge the project staff: Evelyn Silva, Pedro Batista, Melissa LaMar, and Sung-Yeon Kang, for their contributions, and to thank all the participating institutions, recruitment sites, and research participants.

References

1. Kapadia F, Vlahov D, Donahoe RM, Friedland G. The role of substance abuse in HIV disease progression: reconciling differences from laboratory and epidemiologic investigations. *Clin. Infect. Dis.* 2005; 41(7):1027–1034. [PubMed: 16142670]
2. Wang X, Zhang T, Ho WZ. Opioids and HIV/HCV infection. *J. Neuroimmune Pharmacol.* 2011; 6(4):477–489. [PubMed: 21755286]
3. Tomescu C, Seaton KE, Smith P, et al. Innate activation of MDC and NK cells in high-risk HIV-1-exposed seronegative IV-drug users who share needles when compared with low-risk nonsharing IV-drug user controls. *J. Acquir. Immune Defic. Syndr.* 2015; S(3):264–273.
4. Mehendru S, Deren S, Kang SY, et al. Behavioral, mucosal and systemic immune parameters in HIV-infected and uninfected injection drug users. *J. Addict. Res. Ther.* 2015; 6:257.

5. Friedman EM, Christ SL, Mroczek DK. Inflammation partially mediates the association of multimorbidity and functional limitations in a national sample of middle-aged and older adults: the MIDUS study. *J. Aging Health*. 2015; 27(5):843–863. [PubMed: 25649677]
6. Howcroft TK, Campisi J, Louis GB, et al. The role of inflammation in age-related disease. *Aging (Albany NY)*. 2013; 5(1):84–93. [PubMed: 23474627]
7. Mientjes GH, van Ameijden EJ, van den Hoek AJ, Goudsmit J, Miedema F, Coutinho RA. Progression of HIV infection among injecting drug users: indications for a lower rate of progression among those who have frequently borrowed injecting equipment. *AIDS*. 1993; 7(10):1363–1370. [PubMed: 8267910]
8. Donahoe RM, Vlahov D. Opiates as potential cofactors in progression of HIV-1 infections to AIDS. *J. Neuroimmunol*. 1998; 83(1–2):77–87. [PubMed: 9610676]
9. Krol A, Flynn C, Vlahov D, Miedema F, Coutinho RA, van Ameijden EJ. New evidence to reconcile in vitro and epidemiologic data on the possible role of heroin on CD4+ decline among HIV-infected injecting drug users. *Drug Alcohol Depend*. 1999; 54(2):145–154. [PubMed: 10217554]
10. Hagan H. Agent, host, and environment: hepatitis C virus in people who inject drugs. *J. Infect. Dis*. 2011; 204(12):1819–1821. [PubMed: 22013223]
11. Markowitz M, Deren S, Cleland C, et al. Chronic hepatitis C infection and the pro-inflammatory effects of injection drug use. *J. Infect. Dis*. 2016
12. Montgomery RR, Shaw AC. Paradoxical changes in innate immunity in aging: recent progress and new directions. *J. Leukoc. Biol*. 2015; 98(6):937–943. [PubMed: 26188078]
13. R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; url: <http://www.R-project.org/>. 2016
14. Fox J. Effect displays in R for generalised linear models. *Journal of Statistical Software*. 2003; 8(15):1–27.
15. Tran HK, Chartier L, Troung LX, et al. Systemic immune activation in HIV-1-exposed uninfected Vietnamese intravascular drug users. *AIDS Res. Hum. Retroviruses*. 2006; 22(3):255–261. [PubMed: 16545012]
16. Kallas E, Huik K, Turk S, et al. Differences in T cell distribution and CCR5 expression in HIV-positive and HIV-exposed seronegative persons who inject drugs. *Med. Microbiol. Immunol*. 2016; 205(3):231–239. [PubMed: 26696529]
17. Miyazawa M, Lopalco L, Mazzotta F, et al. The 'immunologic advantage' of HIV-exposed seronegative individuals. *AIDS*. 2009; 23(2):161–175. [PubMed: 19098485]
18. Beyrer C, Arstenstein AW, Ruggao S, et al. Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in northern Thailand. *J. Infect. Dis*. 1999; 179(1):59–67. [PubMed: 9841823]
19. Abu-Raya B, Kollmann TR, Marchant A, MacGillivray DM. The immune system of HIV-exposed uninfected infants. *Front. Immunol*. 2016; 7:383. [PubMed: 27733852]
20. Burgener A, Sainsbury J, Plummer FA, Ball TB. Systems biology-based approaches to understand HIV-exposed uninfected women. *Curr. HIV/AIDS Rep*. 2010; 7(2):53–59. [PubMed: 20425558]
21. Batista P, Deren S, Banfield A, et al. Challenges in recruiting people who use drugs for HIV-related biomedical research: perspectives from the field. *AIDS Patient Care STDS*. 2016; 30(8):379–384. [PubMed: 27509238]
22. Appay V, Kelleher AD. Immune activation and immune aging in HIV infection. *Curr. Opin. HIV AIDS*. 2016; 11(2):242–249. [PubMed: 26845675]

Figure 1.
Relationship between injection behaviors and immune activation

Table 1

Characteristics of HIV Seronegative PWID (N = 48)

<i>Socio-demographic characteristics</i>	
Gender (% male)	77.1
Age (mean yrs, sd)	42.5 (7.2)
Race/ethnicity (%)	
Black	14.6
Hispanic	75.0
White	10.4
Education (%)	
< HS	27.1
GED/HS	50.0
> HS	22.9
Homeless (%)	20.8
HCV positive (%)	81.3
HCV viremic	50.0
<i>Risk behaviors</i>	
# years injecting (mean, sd)	16.4 (10.3)
<i>Prior 30 days</i>	
# of injections (mean, sd)	158.4 (200.6)
Shared needles/syringes (%)	20.8
Backloaded (%)	4.2
Shared other paraphernalia (%)	29.2
Any sharing (%)	35.0