

Introduction

60-90% of injection drug users (IDU) in the U.S. who are infected with HIV are coinfecting with hepatitis C virus (HCV). Persons coinfecting with HIV and HCV are at increased risk of progression to severe liver disease and the time course of progression is accelerated compared to persons with HCV alone (Graham, CID, 2001). The prevailing hypothesis for the mechanism of HCV-related liver damage is activation of CD4+ and CD8+ T cells that engage in an ineffective attempt to control the HCV infection. Though polysubstance use is common in HIV/HCV and HCV, it is unclear how IDU and alcohol consumption affect cellular immune responses and thus potentially impact liver disease progression. In this study we hypothesized that IDU and alcohol are associated with immune activation and increased antigen-specific immune responses, and that responses are augmented in those who inject drugs and drink alcohol.

AIMS

To determine whether subjects with active injection drug use, alcohol consumption, or IDU and drinking have increased cellular immune responses compared to those who do not drink alcohol or inject drugs, and if so, what cytokines are affected.

To investigate whether HIV coinfection alters the effect of IDU, alcohol, or both on cellular immune responses.

Study Design

Subjects were recruited from the Boston Medical Center CHARM (Hepatitis C, HIV, And Related Morbidity) cohort, a prospective study that includes extensive demographics, alcohol and drug histories, clinical information, and collection of PBMC every 12 months. Subjects were included in this study if they were 1) current IDU and had not consumed alcohol in at least one year ("IDU"), 2) current alcohol consumers who had not injected drugs in at least one year ("Drinkers"), 3) were current users of IDU and alcohol ("IDU/Drinker"), or 4) if they had not used injection drugs or alcohol in at least one year ("Nonusers"). We studied 16 HIV/HCV IDU, 34 HIV/HCV Drinkers, 24 HIV/HCV IDU/Drinkers, 19 HCV IDU, 19 HCV Drinkers, 14 HCV IDU/Drinkers, 34 HIV/HCV Nonusers, and 19 HCV Nonusers.

ELISPOT Assay

ELISpots were performed on cryopreserved PBMC according to standard techniques. In brief, IP 96-well plates (Millipore Co., Bedford, MA) were coated with 100 µl of primary monoclonal antibody (mAb) (anti-IFN γ or anti-IL-10; Mabtech; anti-TNF α ; Pharmingen) at 10 µg/ml, 2.5 x 10⁶ PBMC in RPMI-1640 were plated in triplicate in the presence of antigens or controls and incubated for 48 hours. The recombinant HCV antigens used were derived from HCV genotype 1b and include core and nonstructural proteins NS3, and NS5 at 1 µg/ml (Mikrogen, Germany). Positive control wells consisted of phytohemagglutinin (PHA, 5 µg/ml, Sigma), Tetanus (3 µg/ml, Accurate) and Candida cellular antigen (20 µg/ml, Greiner Labs, N.C.). Negative control wells were media and buffer used to prepare HCV antigens. Biotin-conjugated secondary mAb were added at 1 µg/ml (IFN γ and IL-10) or 0.5 µg/ml (TNF α) for 2 hours. Plates were developed with avidin-peroxidase and AEC. The numbers of spots per well were scored using a Zeiss reader. Averaged numbers of spot forming cells (SFC) in control wells were subtracted from antigen stimulated wells to correct for spontaneous cytokine production. All values of SFC were normalized per million PBMC. Persons performing ELISPOT assays were blinded to all subject data except PIN and date of sample collection.

Statistical Analysis

Median values between each substance use group and Nonusers were compared for HIV/HCV and HCV groups with the Wilcoxon rank-sum test. In order to determine if IDU status was a similar effect depending on HIV status, cytokine responses > 10 SFC/10⁶ PBMC were scored a priori as "Responders" based on HCV-specific and recall antigen responses in healthy controls. Cochran-Mantel-Haenszel combined odds ratios were calculated as well as the Breslow-Day test for homogeneity.

Table 1: Baseline Demographics

Characteristics	IDU ² N=35	Drinker (N=53)	IDU/Drinker (N=38)	Nonusers N=53
Male sex	54%	70%	76%	70%
Age <40	34%	17%	29%	19%
≥40	66%	83%	71%	64%
Race: White	40%	23%	32%	38%
Black	26%	60%	45%	43%
Hispanic	31%	13%	21%	18%
Other	3%	4%	3%	0%
CD4 count Median, (IQR) ¹	225 (110, 387)	476 (290, 655)	279 (135, 592)	438 (250, 630)
HIV Viral Load <400 copies/ml ¹	45%	79%	42%	59%

¹Data only for HIV-infected subjects. IDU, Drinker and IDU/Drinker groups were each compared to Nonusers and differences with p<0.05 are in bold blue.

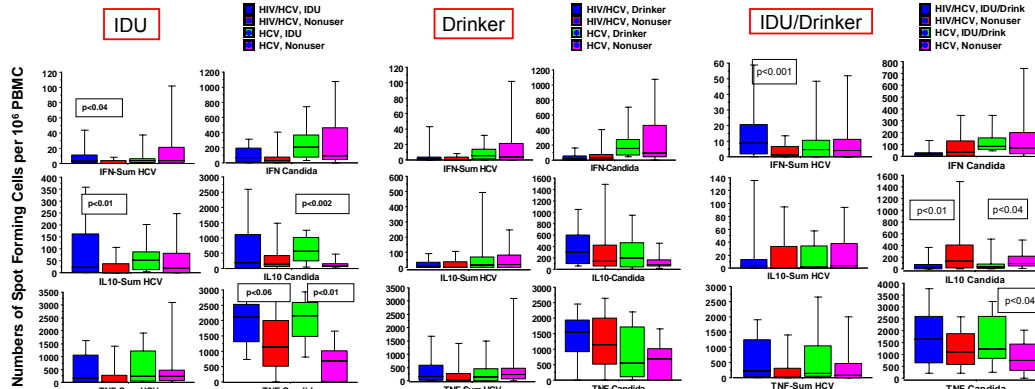
²35% of "Drinkers" and 51% of "IDU/Drinker" drank 5 or more drinks on a typical day, and 27% of Drinkers and 40% of IDU/Drinker had 6 or more drinks on one occasion at least weekly. 94% of IDU¹ and 92% of IDU/Drinker injected drugs 5 or more times per week.

Table 2. IDU Have Higher IL-10 and TNF α Immune Responses while IDU/Drinkers Have Higher TNF α Responses than Nonusers

Cytokine Antigen	IDU (N=35) Median (IQR) ¹	Drinker (N=53) Median (IQR)	IDU/Drinker (N=38) Median (IQR)	Nonusers (N=53) Median (IQR)
IFNγ				
Core	0 (0, 3)	0 (0, 4)	1 (0, 3)	0 (0, 1)
NS3	0 (0, 3)	1 (0, 1)	1 (0, 4)	0 (0, 3)
NS5	0 (0, 1)	1 (0, 1)	1 (0, 11)	0 (0, 1)
Sum-HCV	4 (1, 7)	3 (0, 11)	6 (1, 16)	1 (0, 7)
Candida	156 (41, 281) ²	45 (7, 148)	30 (9, 105)	43 (5, 153)
Tetanus	11 (0, 105)	1 (0, 12)	3 (0, 36)	3 (1, 11)
IL-10				
Core	5 (0, 25)	0 (0, 13)	0 (0, 1)	0 (0, 5)
NS3	4 (0, 21)	0 (0, 4)	0 (0, 5)	0 (0, 21)
NS5	9 (0, 52)	3 (0, 16)	0 (0, 4)	0 (0, 4)
Sum-HCV	43 (8, 106)	7 (0, 44)	2 (0, 15)	0 (0, 65)
Candida	468 (95, 1061)	226 (85, 500)	28 (8, 74)	93 (61, 302)
Tetanus	24 (3, 66)	7 (0, 73)	3 (0, 15)	1 (0, 76)
TNFα				
Core	22 (0, 134)	1 (0, 132)	11 (0, 40)	0 (0, 131)
NS3	15 (0, 288)	5 (0, 117)	44 (0, 451)	0 (0, 162)
NS5	10 (0, 224)	26 (0, 177)	38 (0, 279)	0 (0, 36)
Sum-HCV	210 (30, 1205)	138 (0, 558)	138 (32, 1251)	87 (0, 424)
Candida	2132 (1318, 2572)	1430 (345, 1738)	1439 (650, 2669)	880 (412, 1728)
Tetanus	123 (18, 1043)	55 (0, 343)	131 (34, 355)	34 (0, 491)

¹Numbers of SFC/10⁶ PBMC. ²IDU, Drinkers and IDU/Drinkers each compared to Nonusers, and all comparisons with p<0.05 are in bold blue.

Figure 1. Comparison of Antigen-specific Responses in Subjects with HIV/HCV versus HCV who are IDU, Drinkers, or IDU/Drinkers versus Nonusers



Immune responses in HIV/HCV substance users (blue = IDU, Drinker, or IDU/Drinker) were compared to HIV/HCV Nonusers (red), and HCV substance users (green) were compared to HCV Nonusers (pink), to determine whether differences in immune responses were attributable to HIV/HCV groups, HCV groups, or both. Significant differences are indicated.

Table 3. Cochran-Mantel-Haenszel Odds Ratios for Effect of IDU, Drinkers, or IDU/Drinker Controlling for HIV Status

Cytokine Antigen	IDU OR (95% CI)	Drinkers OR (95% CI)	IDU/Drinkers (95% CI)
IFNγ			
Core	*	*	0.94 (0.23, 3.86)
NS3	1.64 (0.37, 7.35)	*	1.43 (0.43, 4.71)
NS5	*	(both groups = 0)	5.07 (1.63, 15.73)
Candida	2.59 (0.82, 8.16)	1.49 (0.62, 3.66)	1.46 (0.59, 3.66)
Tetanus	2.49 (0.81, 7.66)	*	2.97 (1.14, 7.78)
IL-10			
Core	2.50 (0.95, 6.56)	1.37 (0.56, 3.38)	1.00 (0.42, 2.40)
NS3	1.00 (0.40, 2.51)	0.24 (0.09, 0.68)	0.75 (0.25, 2.23)
NS5	2.95 (1.14, 7.68)	1.96 (0.82, 4.69)	0.91 (0.37, 2.23)
Candida	1.13 (0.27, 4.72)	1.22 (0.35, 4.28)	0.87 (0.31, 2.46)
Tetanus	2.76 (1.08, 7.06)	1.26 (0.58, 2.74)	0.45 (0.18, 1.34)
TNFα			
Core	1.71 (0.69, 4.23)	1.55 (0.70, 3.42)	2.58 (1.07, 6.26)
NS3	1.29 (0.53, 3.11)	1.03 (0.48, 2.24)	2.76 (1.11, 6.87)
NS5	1.50 (0.61, 3.70)	*	6.60 (2.42, 17.98)
Candida	9.82 (1.03, 93.90)	0.98 (0.35, 2.73)	3.57 (0.43, 29.95)
Tetanus	3.08 (1.08, 8.80)	0.97 (0.45, 2.10)	5.14 (1.60, 16.54)

Here, Responders were defined as having >10 SFC/10⁶ PBMC and Responders were compared to Nonresponders for HIV/HCV Users versus Nonusers and separately for HCV Users versus Nonusers for each group: IDU, Drinkers, and IDU/Drinkers (data not shown). Then, combined odds ratios for likelihood of responding were calculated, as presented in Table 3. This C-M-H odds ratio represents the "effect" due to IDU, alcohol, or IDU/alcohol when controlling for HIV.

^{*}Combined OR were not performed ("") because the p value for the Breslow-Day test of homogeneity was <0.05. This is consistent with HIV acting as an effect modifier, so it is not appropriate to combine groups.

Conclusions

•Subjects with injection drug use had higher IL-10 and TNF α HCV specific and recall antigen responses compared to Nonusers (Table 2). Higher IFN γ and IL-10 HCV-specific responses appeared to be driven by HIV/HCV IDU, while higher IL-10 and TNF α recall antigen responses were seen in HCV IDU (Figure 1).

•In contrast, alcohol had modest effects on IL-10 responses (Table 2) and no immune responses differences were found when subjects were split by HIV status (Figure 1).

•IDU/Drinker status had the most prominent effect on antigen-specific TNF α secretion, though, as with HIV/HCV IDU, the HIV/HCV IDU/Drinkers had higher HCV-specific IFN γ secretion.

•When controlling for HIV status, IDU was associated with significantly higher IL-10 responses as well as TNF α recall responses while IDU/Drinker exposure was associated with broadly increased TNF α responses.

•Overall, antigen-specific IFN γ responses seem most differentially affected by HIV status (higher IFN γ in HIV/HCV IDU or drinkers, and lower in HCV IDU or drinkers with significant Breslow Day test), while IFN γ responses are higher in both groups in IDU/Drinkers (Table 3), substance use needs to be taken into account when studying HCV-specific IFN γ immune responses.