Influence of Hepatitis C Virus Sustained Virological Response on Immunosuppressive Tryptophan Catabolism in ART-Treated HIV/HCV Coinfected Patients

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Background: We previously reported an association between tryptophan (Trp) catabolism and immune dysfunction in HIV monoinfection. Coinfection with HIV is associated with more rapid evolution of hepatitis C virus (HCV)–associated liver disease despite antiretroviral therapy (ART), possibly due to immune dysregulation. We hypothesized that liver fibrosis in HIV/HCV coinfection would be associated with immune dysfunction and alterations in Trp metabolism.

Methods: Trp catabolism and inflammatory soluble markers were assessed in plasma samples from ART-treated HIV/HCV-coinfected patients (n = 90) compared with ART-treated HIV-monoinfected patients and noninfected subjects. Furthermore, 17 additional coinfected patients with sustained virological response (SVR) were assessed longitudinally 6 months after completion of interferon-α/ribavirin treatment.

Results: HIV/HCV patients had higher Trp catabolism compared with HIV-monoinfected and healthy individuals. Elevated kynurenine levels in HIV/HCV patients with liver fibrosis correlated with the prognostic aspartate aminotransaminase to platelet ratio (APRI scores) and insulin levels. Furthermore, HIV/HCV patients had elevated levels of disease progression markers interleukin-6 and induced protein 10 and shared similar levels of markers of microbial translocation (intestinal fatty acid-binding protein, soluble CD14 and lipopolysaccharide-binding protein) compared with HIV-monoinfected and healthy individuals. Successful HCV treatment improved APRI score and markers of disease progression and microbial translocation although elevated Trp catabolism remained unchanged 6 months after SVR.

Conclusion: ART-treated HIV/HCV-coinfected patients had elevated immunosuppressive Trp catabolism when compared with monoinfected HIV-treated patients, which did not normalize after SVR. These findings suggest that a necroinflammatory liver syndrome persists through inflammation by Trp catabolism after 6 month of SVR.

Key Words: indoleamine 2,3-dioxygenase 1, tryptophan, HIV, HCV, co-infection, inflammation

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INTRODUCTION

Because of shared routes of transmission, HIV and hepatitis C virus (HCV) coinfection is an important public health problem. An estimated 5–7 million individuals are afflicted with both infections worldwide and in the United States, one-quarter of HIV-infected individuals are estimated to be HCV coinfected.[1,2] Many deleterious effects of HIV on the natural history of HCV are well documented. Spontaneous HCV clearance rates are 20% in individuals without HIV-coinfection, contrasting with 5%–10% in those coinfected.[2–4] HCV RNA levels are higher in coinfected compared with monoinfected individuals.[5] HIV/HCV coinfection has been associated with faster rates of fibrosis, greater risk of liver failure resulting in death, and suboptimal responses to HCV therapy compared to individuals with HCV monoinfection.[2,6,7] Importantly, these deleterious squeals do not seem to be completely attenuated by antiretroviral therapy (ART).[2,8,9]

A hallmark of HIV infection is marked reduction in CD4 T cells in gut-associated lymphoid tissue.[10] HIV is also characterized by gut mucosal leakiness, enabling bacterial translocation of lipopolysaccharide (LPS) and other microbial products resulting in chronic immune activation.[11] Individuals with HIV/HCV coinfection also demonstrate increased amounts of bacterial translocation, as demonstrated by high

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levels of LPS-binding protein and monocyte and lymphocyte activation markers compared with uninfected healthy controls.[12] Furthermore, markers of microbial and immune activation, such as sCD14 and interleukin (IL)-6, are increased in HIV/HCV-coinfected patients with hepatic cirrhosis compared with those without cirrhosis.[12] The liver plays an important role in bacterial clearance through the hepatic venous circulation, which is increasingly impaired as liver disease advances.[13] Attempting to discern the relative contribution and the interaction between HIV versus HCV-associated immune dysfunction and microbial translocation is an important goal.

In recent years, attention has been drawn to the pivotal role of indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme catabolizing tryptophan (Trp) to kynurenine (Kyn). Trp catabolism is involved in a wide variety of immune responses aimed at protecting the individuals from over-reactive immune reactions through the induction of systemic immune tolerance.[14] By modulating dendritic cell maturation, Trp catabolism results in depletion of T cells and expansion of Tregs.[15] In HIV infection, high IDO activity and Trp catabolism is associated with reduced antiviral T-cell responses and increase in Tregs frequency resulting in enhanced mortality.[16–19] In HIV monoinfection, our group demonstrated that IDO-induced Trp catabolism into Kyn results in disruption of the Th17/Treg ratio that facilitates microbial translocation.[19–21] Furthermore, we showed a distinctive Trp catabolism and Th17/Treg balance in HIV progressors compared with elite controllers, suggesting an important role for Trp catabolism in HIV pathogenesis.[21] Trp catabolism has also been shown to play a key role in chronic HCV infection.[22] High intrahepatic IDO upregulation and increased serum Kyn levels have been documented in individuals with chronic HCV infection[22] contributing to Treg induction.[23] Furthermore, insulin resistance has been proposed to be linked with Trp catabolism in HCV infection.[24] High Trp catabolism has also been demonstrated within the livers of individuals with HCV-induced liver cirrhosis.[25] Indeed, all these data suggest an important role for IDO in both HIV and HCV monoinfection.

To date, little is known about immunosuppressive Trp catabolism in the context of HIV/HCV coinfection and specifically whether cure of HCV can restore abnormal Trp catabolism. We therefore assessed Trp catabolism and the impact of liver fibrosis in ART-treated HIV/HCV-coinfected patients before and after HCV sustained virological response (SVR).

MATERIALS AND METHODS

Study Population

We used plasma samples from HIV/HCV-coinfected individuals successfully treated with ART that were collected prospectively through the prospective Canadian Coinfection Cohort[26] and stored frozen and never thawed before our analyses. Diagnosis of HIV infection was established based on a positive enzyme-linked immunosorbent assay (ELISA) and/or a detectable HIV RNA and confirmed by Western blot. HCV infection was established based on HCV seropositive tests by ELISA with recombinant immunoblot assay II or enzyme immunoassay confirmation or detectable HCV RNA in cases of serological false negatives. At visits every 6 months, socio-demographic, clinical and behavioral information including alcohol abuse was collected using validated questionnaires. We randomly selected 90 from patients with evidence of chronic HCV infection [HCV RNA positive by Roche Amplicor 2.0 assay (Roche Diagnostics, Indianapolis, IN), dynamic range: 600–500,000 IU/mL] on ART with suppressed HIV RNA (<1.6 log copies/mL, Abbott HIV-1 RealTime m2000rt Abbott Molecular Inc., Des Plaines, IL). We used the validated noninvasive surrogate marker of liver fibrosis aspartate aminotransaminase to platelet ratio (APRI score) to categorize patients as having significant liver fibrosis based on a score greater than or less than/equal to 1.5.[27] An APRI score below 0.5 was used to determine the absence of fibrosis. A longitudinal assessment was also performed on a second group of HIV/HCV-coinfected patients in the Canadian Coinfection Cohort who achieved SVR after treatment with pegylated-interferon (IFN) ribavirin at 2 time–points: 12 months before treatment and 6 months after SVR determination. Samples from ART-treated HIV-monoinfected subjects and healthy subjects were obtained from the Chronic Viral Illness Service, McGill University Health Centre (MUHC), Montreal, QC, Canada [healthy subjects (HS)] (Table 1).

Ethics Statement

This study was conducted according to the Declaration of Helsinki and received approval from the MUHC's Ethical Review Board and the ethics boards of all participating sites. All patients and HS provided written informed consent for their participation in the study.

Measurement of the IDO Enzymatic Activity

Plasma levels of Trp and its catabolite Kyn were measured by an automated online solid-phase extraction-liquid chromatographic-tandem mass spectrometric method as previously reported.[20,21,28] The IDO enzymatic activity was determined by plasma Kyn/Trp ratio.

Insulin Quantification

Plasma levels of insulin were quantified according to the manufacturer's instructions using Unicel DxI 800 analyzer and reagent (Access immunosystems Insulin; Beckman Coulter, Brea, CA).

Measurement of Plasma Levels of the Markers of Inflammation and Microbial Translocation

Plasma levels of the markers of inflammation IFN gamma-induced protein 10 (IP-10), also known as C-X-C motif chemokine 10, and interleukin (IL)-6 were measured by ELISA using commercially available kits from R&D system (Minneapolis, MN). Plasma levels of markers of intestinal mucosal damage, intestinal fatty acid-binding

TABLE 1. Clinical Characteristics of Study Groups

	Study Population (N = 133)					
	ART-Treated HIV/HCV Coinfected (n = 90)		Longitudinal HIV/HCV Coinfected Before and After SVR		Control Groups	
Characteristics	Fibrotic (n = 44)	Nonfibrotic (n = 46)	Precure (n = 17)	Postcure (n = 17)	ART-Treated HIV-Monoinfected (n = 14)	Healthy Subjects (n = 12)
Age [mean ± SD (range)], yrs	46 ± 8 (26–61)	46 ± 7 (26–62)	46 ± 8 (31–60)	46 ± 8 (31–60)	46 ± 8 (30–57)	48 ± 8 (35–60)
Male, n (%)	36 (82)	36 (78)	15 (88)	15 (88)	12 (86)	7 (58)
CD4 T-cell count [mean ± SD (range)], cells/μL	510 ± 216 (103–1370)	557 ± 164 (370–971)	482 ± 267 (76–1032)	525 ± 180 (163–849)	528 ± 155 (267–866)	869 ± 306 (281–1360)
CD8 T-cell count [mean ± SD (range)], cells/μL	754 ± 469 (296–2408)	945 ± 822 (350–6000)	964 ± 571 (260–2561)	793 ± 321 (264–1392)	855 ± 397 (466–2013)	470 ± 178 (227–843)
CD4:CD8 ratio [mean ± SD (range)]	0.83 ± 0.41 (0.25–1.90)	0.74 ± 0.35 (0.15–1.70)	0.59 ± 0.33 (0.05–1.57)	0.75 ± 0.33 (0.12–1.63)	0.73 ± 0.41 (0.24–1.86)	2.11 ± 0.99 (0.38–3.97)
HCV viral load [mean ± SD (range)], log10 copies/mL	6.20 ± 6.31 (4.32–6.75)	7.73 ± 8.24 (0–8.78)	7.03 ± 7.33 (3.41–7.73)	NA	NA	NA
Duration of HCV infection [mean ± SD (range)], yrs	20.9 ± 10.33 (0.6–50.5)	21.6 ± 9.4 (2.3–1.4)	20 ± 13.4 (0.4–40.6)	20.39 ± 12.22 (1.8–42)	NA	NA
Duration of HIV infection [mean ± SD (range)], yrs	13.8 ± 6.64 (1.8–31.2)	15.3 ± 6.4 (3.7–28.9)	11.8 ± 4.9 (2.8–18.8)	13.2 ± 4.9 (4.6–20.9)	9.3 ± 4.1 (2.7–16.2)	NA
Time since start of ART [mean ± SD (range)], yrs	9.3 ± 4.9 (1.7–20.1)	9.5 ± 5.5 (1.8–22.7)	7.5 ± 4.6 (0.4–16.2)	9.4 ± 5.1 (1.8–18.1)	8 ± 3 (4.6–14.2)	NA
APRI score [mean ± SD (range)]	2.79 ± 2.24 (1.61–14.94)	0.35 ± 0.09 (0.18–0.49)	0.86 ± 0.51 (0.17–2.09)	0.39 ± 0.18 (0.12–0.99)	NA	NA
Fibroscan score* [mean ± SD (range)]	19.63 ± 19.43 (5.40–69.10)	5.63 ± 1.48 (3.70–9.30)	11.80 (11.80–1.80)	9.35 ± 5.24 (4.40–19.00)	NA	NA

Results are shown as mean ± SD and (range).
*Fibrosis stage using transient elastography (FibroScan TM) on a subset of patients (10 in fibrotic and 15 in nonfibrotic groups) from centers where this was available.
NA, not applicable.

protein (I-FABP) and soluble CD14 (sCD14), were measured by ELISA using commercially available kits from Hycult Biotech (Uden, the Netherlands) and R&D system, respectively. Plasma levels of the marker of microbial translocation, lipopolysaccharide-binding protein (LBP), were measured using commercially available ELISA kits from Hycult Biotech.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism software version 5. Kruskal–Wallis tests were performed for comparisons between study groups. Unpaired *t* tests were used for comparisons of 2 nonpaired study variables. Wilcoxon matched pairs test was used for comparisons of 2 paired study variables. The Pearson rank correlation test was used to identify associations among study variables.

RESULTS

Study Population

Ninety patients with HIV/HCV coinfection were studied. Patients were subdivided into either fibrotic (n = 44) or nonfibrotic (n = 46) groups based on APRI score. We were able to confirm fibrosis stage using transient elastography (FibroScan TM) on a subset of patients (10 in fibrotic and 15 in nonfibrotic groups) from centers where this was available (Table 1). On average, those in the fibrotic group had advanced fibrosis/cirrhosis with mean APRI scores corresponding to F3-F4 on liver biopsy. No difference was observed in alcohol use or abuse between the 2 groups.

Seventeen additional coinfected patients were also sampled longitudinally 12 months before treatment with IFN-α/ribavirin and 6 months after having shown a SVR. Patient clinical characteristics are described in Table 1.

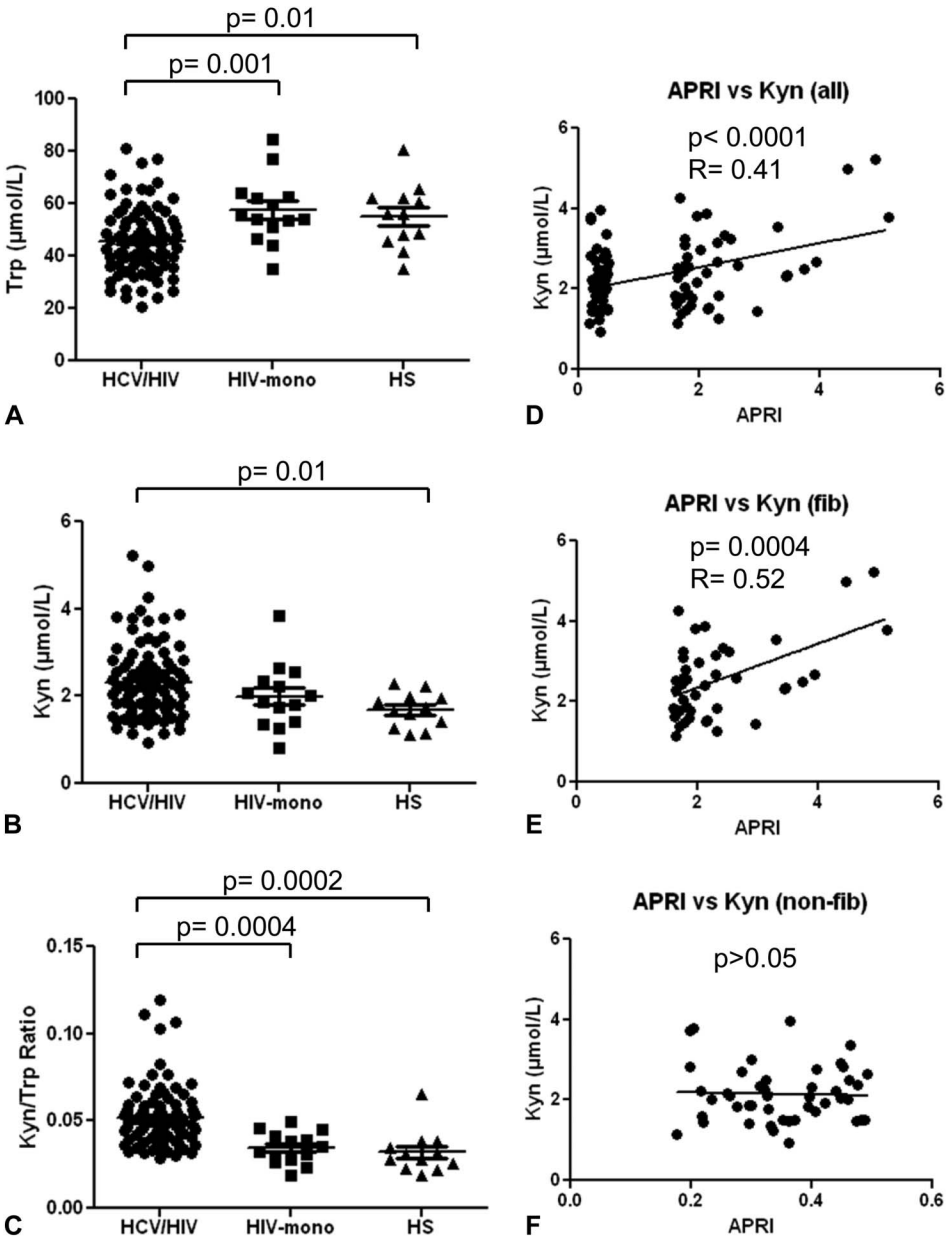

FIGURE 1. Immunosuppressive Trp catabolism is higher HIV/HCV coinfection in association with liver fibrosis. Plasma levels of (A) Trp, (B) Kyn, and (C) IDO enzyme activity defined as the Kyn/Trp ratio in HIV/HCV-coinfected, HIV-monoinfected, and HS. Association between plasmatic levels of immunosuppressive Kyn and APRI scores in (D) all HIV/HCV-coinfected subjects and (E) patients with liver fibrosis or (F) without liver fibrosis.

Immunosuppressive Kyn and Kyn/Trp Ratio Are Elevated in HIV/HCV Coinfection in Association With Liver Disease Progression Independently of HCV Viremia

Patients with HIV/HCV coinfection displayed decreased levels of Trp compared with both HIV-monoinfected and HS (45.76 ± 12.44 vs 57.52 ± 12.75 and 55.13 ± 12.28 µmol/L, respectively, Fig. 1A). However, patients displayed increased Kyn levels compared with HIV-monoinfected and HS (2.33 ± 0.86 vs 2.00 ± 0.74 and 1.68 ± 0.40 µmol/L, respectively, Fig. 1B). Accordingly, coinfected patients displayed increased Kyn/Trp ratios, the plasma indicator of IDO activity, compared with HIV-monoinfected and HS (0.052 ± 0.017 vs 0.035 ± 0.009 and 0.032 ± 0.012, respectively, Fig. 1C).

Among coinfected individuals, Kyn levels were primarily elevated in fibrotic patients (2.52 ± 0.97 vs 2.15 ± 0.70 µmol/L in comparison to nonfibrotic patients, $P = 0.04$). This finding was not affected by fasting state at the time of blood draw ($P > 0.05$, data not shown). When examining the association between liver disease progression as measured by APRI score and Kyn levels, a significant correlation was found between coinfected patients and Kyn levels (R = 0.41, $P < 0.0001$, Fig. 1D). When patients were broken down into groups, this correlation was seen only in fibrotic (R = 0.52, $P = 0.0004$, Fig. 1E) but not nonfibrotic patients (Fig. 1F). We have previously shown an association between HIV viral load and Trp catabolism and Kyn plasma levels.[21,28] Unlike HIV viremia, level of HCV viremia was not correlated with Trp catabolism (data not shown).

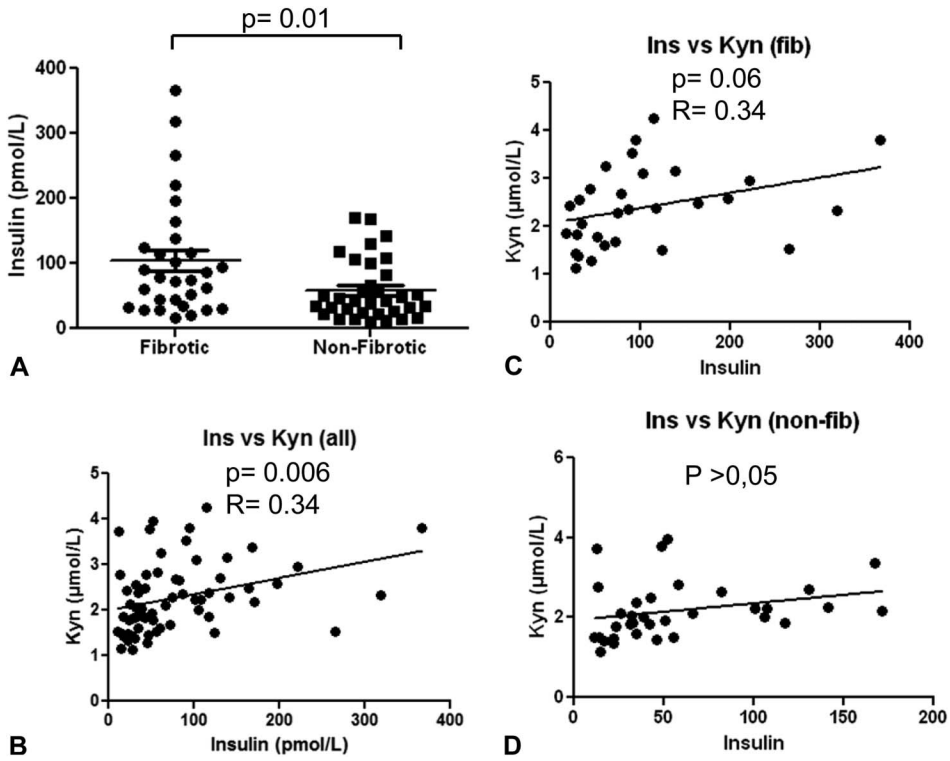

FIGURE 2. Insulin levels are elevated in fibrotic patients and correlate with Kyn levels. A, Plasma levels of insulin in samples from fasted patients with liver fibrosis or without liver fibrosis. Association between plasmatic levels of immunosuppressive Kyn and insulin in (B) all HIV/HCV-coinfected subjects and (C) patients with liver fibrosis or (D) without liver fibrosis.

Insulin Levels Are Elevated in Fibrotic Patients and Correlate With Kyn Levels

As insulin resistance is linked with Trp catabolism in HCV infection,[24] we also assessed the association between plasma insulin and Kyn levels in a subset of specimens for which we had access to the samples from fasted study subject. In plasma samples obtained from fasted patients, insulin levels were increased in patients with fibrotic livers in comparison to nonfibrotic HIV/HCV-coinfected patients (103.7 ± 89.56 vs 57.86 ± 45.62 pmol/L, $P = 0.01$, Fig. 2A). We also observed a modest correlation between insulin and Kyn levels overall among coinfected patients (Fig. 2B). This correlation seemed to be related to the presence of fibrosis (Figs. 2C, D).

Inflammatory Markers of HIV and HCV Disease Progression, But Not Markers of Microbial Translocation, are Increased in Coinfection and Associated With Trp Catabolism

We assessed 2 inflammatory markers of HIV and HCV disease progression. IP-10 was notably evaluated in co-infection when compared to HIV-monoinfected and HS (352.4 ± 361.4 vs 133.5 ± 143.3 and 122.6 ± 80.98 pg/mL, respectively, Fig. 3A). When assessed based on liver fibrosis, although both groups had higher IP-10 levels than HIV-monoinfected or HS, IP-10 was more elevated in the fibrotic compared with nonfibrotic groups (484.3 ± 475.5 vs 244.4 ± 171.7 pg/mL, $P = 0.002$). IP-10 levels were correlated with Kyn levels (Fig. 3D). In contrast, IL-6 levels in co-infection were elevated to a similar degree as those seen in HIV-monoinfected patients; both were higher than HS (5.64 ± 5.46 and 3.95 ± 1.95 vs 2.31 ± 1.79 pg/mL, respectively, Fig. 3B). There was no difference between fibrotic and nonfibrotic groups in IL-6 levels (6.15 ± 5.29 vs 5.10 ± 5.65 pg/mL). IL-6 was also weakly correlated with Kyn in coinfection ($R = 0.23$, $P = 0.04$ Fig. 3E). Levels of I-FABP, LBP, and sCD14 were similarly higher in coinfected and monoinfected individuals compared to HS (Figs. 3C, G, H). No correlation was found between I-FABP and Kyn levels (Fig. 3F). However, a correlation was found between sCD14 and Kyn levels (Fig. 3I) and between LBP and Kyn levels during coinfection (Fig. 3J).

Successful HCV Treatment Did Not Impact the Elevated Trp Catabolism 6 Months After SVR

Patients with SVR displayed a decrease in APRI score (0.86 ± 0.51 vs 0.39 ± 0.18, Fig. 4A; Table 1) 6 months after treatment. When assessed longitudinally, no change was found for Kyn levels (2.23 ± 0.56 vs 2.08 ± 0.42 µmol/L, $P > 0.5$, Fig. 4B) or Kyn/Trp ratios (0.046 ± 0.012 vs 0.042 ± 0.011, $P > 0.5$, Fig. 4C). However, a significant decrease was found for both IP-10 (388.5 ± 461.0 vs 134.3 ± 82.4 pg/mL, $P = 0.0009$, Fig. 4D) and IL-6 (4.62 ± 2.89 vs 2.97 ± 1.57 pg/mL, $P = 0.008$, Fig. 4E). Intestinal damage, as measured by I-FABP, also decreased (1127 ± 866 vs 754 ± 609 pg/mL, $P = 0.02$, Fig. 4F). Although sCD14 levels did not change (1681 ± 389 vs 1564 ± 370 ng/mL, Fig. 4G),

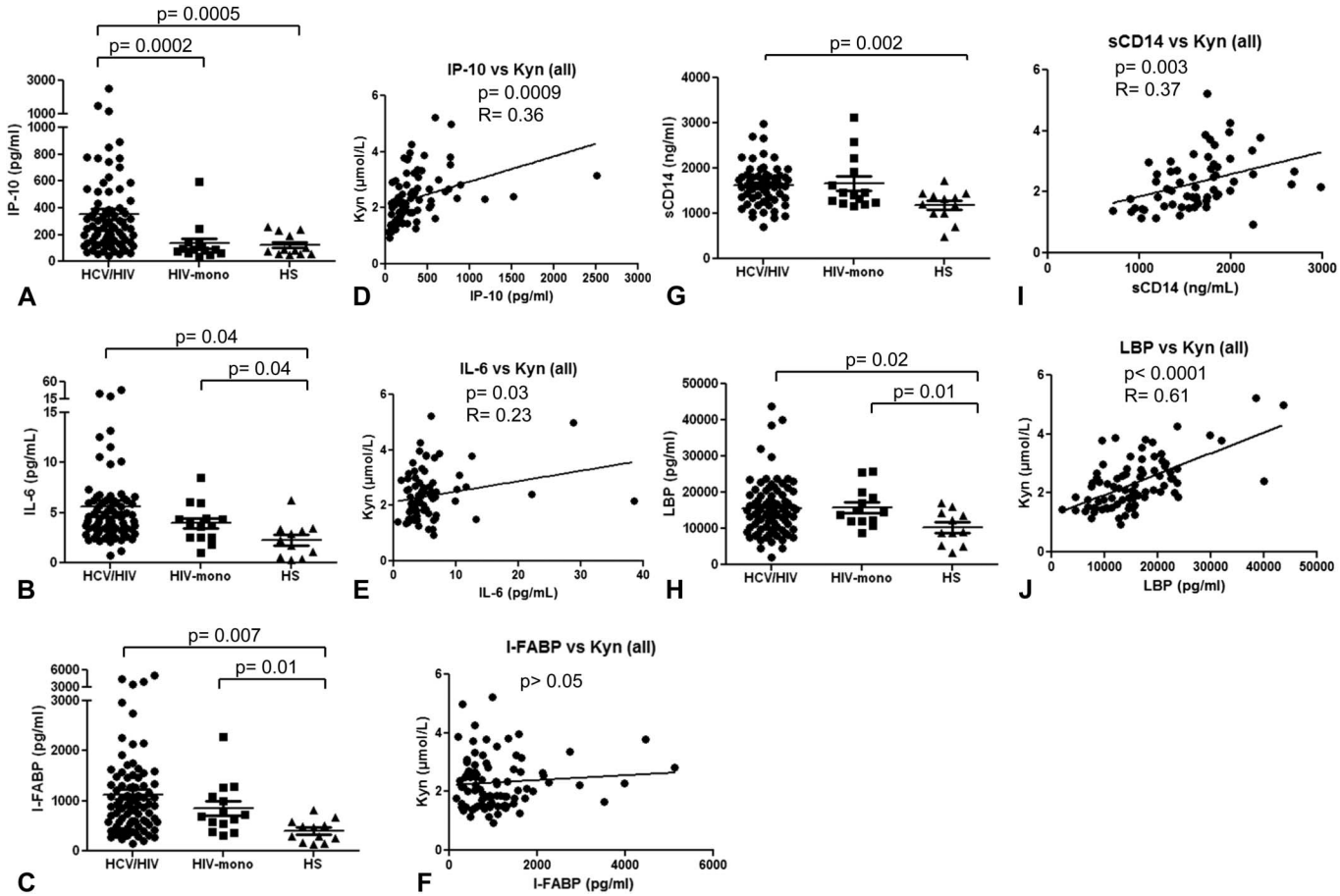

FIGURE 3. Inflammatory markers of HIV and HCV disease progression, but not markers of microbial translocation, are increased in coinfection and associated with Kyn levels. Plasma levels of (A) IP-10, (B) IL-6, (C) I-FABP, (G) sCD14, and (H) LBP in HIV/HCV-coinfected, HIV-monoinfected, and HS and their correlation with plasmatic levels of immunosuppressive Kyn (D–F, I, and J, respectively) in HIV/HCV-coinfected subjects.

levels of LBP did drop (16,902 ± 7595 vs 13,464 ± 6682 pg/mL, $P = 0.05$, Fig. 4H).

DISCUSSION

HIV–HCV coinfection is associated with increased rates of liver disease and risk of mortality compared with HCV monoinfection.[2,29] It has been shown that systemic Trp catabolism is enhanced in HCV monoinfected patients in association with liver inflammation and fibrosis and contributes to immune dysfunction.[22,23] Furthermore, the production of Kyn, the immunosuppressive catabolite of Trp, is related to diverse inflammatory response such as inflammatory aspects of coronary events.[30] Our group has previously shown that Kyn production through Trp catabolism is involved in T cell dysfunction and generalized immune activation and microbial translocation in HIV-monoinfected patients.[19,21,28] We later demonstrated that early initiation of ART was able to reduce Trp catabolism and immune activation in HIV-monoinfected individuals.[28] In addition, Trp catabolism is recognized as an independent predictor of HIV disease progression and mortality.[18,31] In this study, we extended our evaluation to individuals with HIV/HCV-coinfection to assess the association between hepatic fibrosis and Trp catabolism. We found that ART-treated HIV/HCV-coinfected individuals had higher levels of Trp catabolism compared with ART-treated HIV-monoinfected individuals and HS.

Importantly, we observed that liver fibrosis seemed to be the primary driver of altered Trp catabolism suggesting that hepatic inflammation and histological damage associated with HCV rather than the HCV virus itself can favor immunosuppressive Trp catabolism. In addition to IDO, Trp can also be catabolized by another enzyme mainly expressed in the liver, the tryptophan 2,3-dioxygenase (TDO).[32] It has been shown that TDO is expressed in various human tumors and is equally capable of suppressing antitumor immune responses.[33] It is possible that hepatic inflammation and fibrosis can induce and activate TDO, resulting in a higher Trp catabolism in HIV/HCV-coinfected patients. We have shown previously a strong correlation between HIV viral load and Trp catabolism, which can be rapidly normalized by ART[21,28] as HIV Tat protein is directly involved in the induction of IDO enzyme and Trp catabolism.[34] In contrast to HIV viral load and in line with a previous study,[23] we

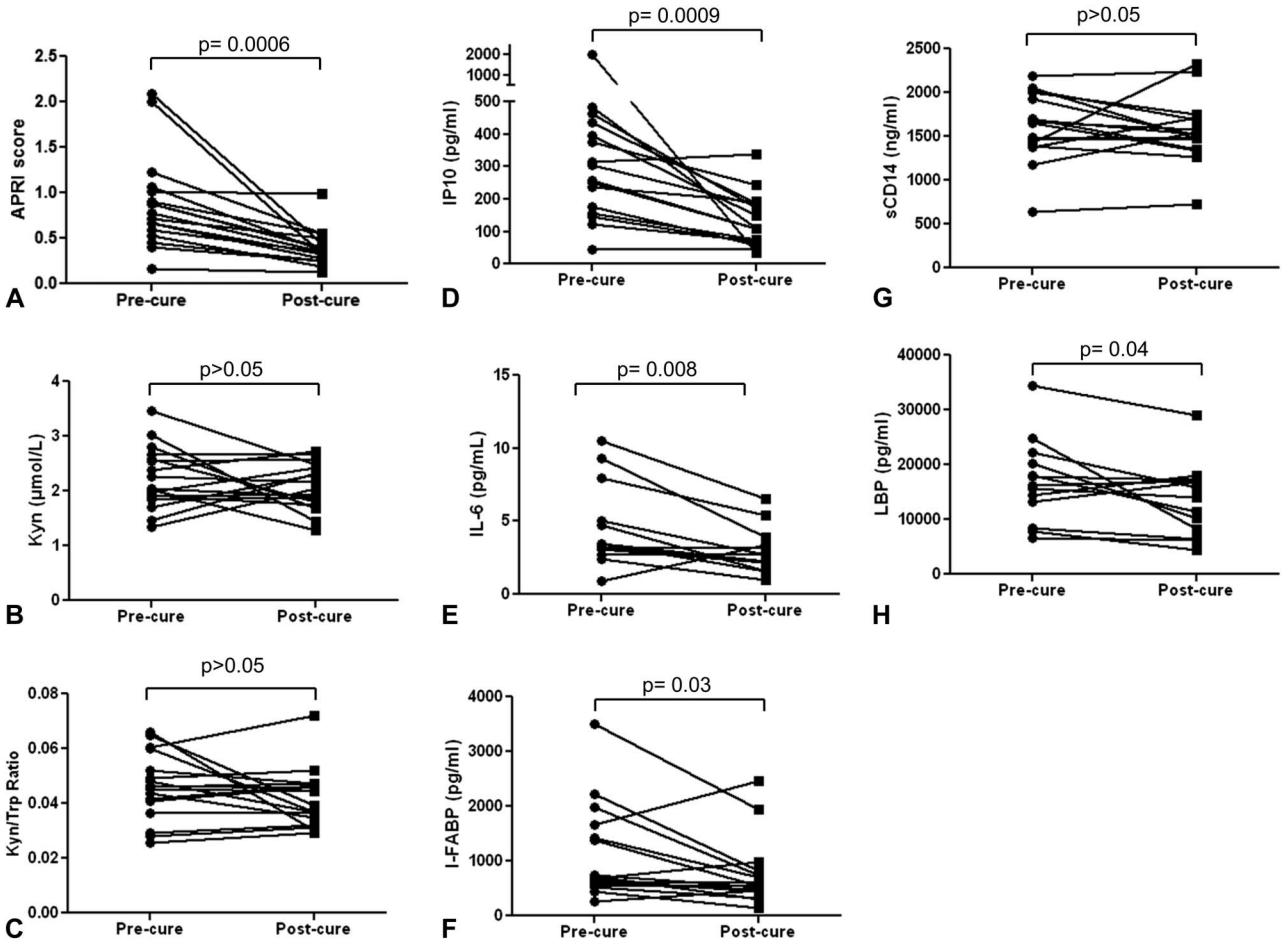

FIGURE 4. Influence of HCV SVR on immunosuppressive Trp catabolism in ART-treated HIV/HCV-coinfected patients. A, APRI score and levels of (B) Kyn, (C) Kyn/Trp ratios, (D) IP-10, (E) IL-6 (F) I-FABP, (G) sCD14, (H) LBP sampled longitudinally 12 months before treatment with IFN-α/ribavirin (precure) and 6 months after, having shown a SVR (postcure).

observed that Trp catabolism was not associated with HCV viral load. The lack of an association with HCV viral replication further supports that fibrosis is the principle factor accounting for higher Trp catabolism in this setting.

There is some evidence that HCV may induce insulin resistance because there is a correlation between degree of insulin resistance and levels of HCV replication, and improvement in insulin resistance after SVR has been reported.[35,36] Furthermore, HCV may interfere with the insulin signaling pathway through proteasomal degradation of the insulin receptor substrate 1 and 2 or by increasing levels of proinflammatory TNF-α.[37] HIV itself seems also to be involved in long-term metabolic changes including insulin resistance.[38] Importantly, insulin resistance is linked with Trp catabolism in HCV infection as an association has been previously observed with homeostatic model assessment index and Kyn plasma levels.[24] Interestingly, we observed that higher levels of fasting plasma insulin in coinfected patients were associated with Trp catabolism in our cohort. Again, this finding was associated with fibrosis stage suggesting that liver fibrosis in coinfected patients can have a negative impact on regulation of Trp catabolism and development of insulin resistance. Furthermore, patients with HCV monoinfection or HIV/HCV-coinfection have neurocognitive disorders. One potential consequence of altered Trp catabolism in the setting of HIV/HCV is that it could contribute to the higher rates of neurocognitive symptoms that have been observed among coinfected persons. Indeed, Trp breakdown by Kyn pathway is directly involved in neurocognitive disorders in HIV infection.[39] However, it has been recently shown that depression is not associated with peripheral insulin resistance in chronically HCV-infected patients.[40] Furthermore, low Trp levels have been linked with high frequency of psychopathology in HCV patients.[41] This highlights the potential influence of liver fibrosis in the development of neurocognitive disorders in HIV/HCV coinfected patients through Trp catabolism.

We have previously shown that Trp catabolism was associated with levels of markers of HIV disease progression IL-6 and IP-10.[21,28] These markers are also known predictors of disease progression and liver disease in HCV monoinfection and HIV/HCV-coinfection.[42–45] Our results show a correlation between Trp catabolism and levels of IL-6 and IP-10. Importantly, levels of IP-10 were higher in HIV/HCV-coinfected

patients compared with HIV-monoinfected patients and HS while they normalized in ART-treated HIV-monoinfected patients. Of note, both IDO enzyme and IP-10 are induced by IFN and it has been shown that DCs from HCV monoinfected patients induce more IDO in response to LPS and IFNγ compared to HS.[23] It has been suggested that HIV may contribute to the development of hepatic fibrosis through microbial translocation.[2,29,38] Indeed, increased LPS due to gut mucosal damage can activate hepatic stellate cells while Kupffer cells express Toll-like receptor 4 and are very responsive to low quantities of LPS.[46,47] This, in turn, results in the release of mediators driving hepatic fibrosis, such as TGF-β1, TIMP-1, and type 2 collagen.[2,29] We therefore assessed the markers of gut mucosal damage (I-FABP) and microbial translocation (LBP and sCD14). We observed similar levels of these markers in HIV/HCV-coinfected and HIV-monoinfected patients. However, strong correlations were observed between levels of LBP and sCD14 and Trp catabolism. Indeed, strong correlation between Kyn and LBP plasma levels as a marker of microbial translocation highlights the potential contribution of histopathological damages and liver clearance capacity on the expression of these inflammatory markers. This could suggest that, because of the chronic hepatic inflammation and Kupffer cell dysfunction, hepatic clearance of microbial products decreases and the liver is unable to maintain its role as a "fire-wall." In line with these findings, we previously observed a correlation between microbial translocation and Trp catabolism because of an altered balance between Th17/Treg cells in HIV monoinfection.[21] Of note, we have correlations observed for Kyn plasma levels, which were not always consistent with the correlations for Kyn/Trp ratios. In addition, in this study, we do not have the information about antibiotic usage or gastrointestinal symptoms in study subjects that could have an impact on the markers of gut mucosal damage.

Finally, in an additional cohort of coinfected patients, we longitudinally assessed the influence of HCV cure on inflammatory markers 6 months after SVR. We observed a significant decrease in APRI score, a surrogate marker of liver fibrosis. Accordingly, the levels of IP-10, IL-6, I-FABP, and LBP were significantly decreased after SVR while sCD14 levels remained unchanged. In line with our results, it has been recently shown that microbial translocation, notably LBP but not sCD14, are associated with SVR after anti-HCV treatment in HIV/HCV-coinfected patients.[48] Importantly, no change was observed in Trp catabolism 6 month after SVR. These findings suggest that, despite the improvement of in the marker liver fibrosis, recovery of tissue damage may lag and metabolic syndromes associated with a necroinflammatory liver syndrome persist even after SVR. It should be stated that IDO is an IFN-induced enzyme and IFN-based treatment could favor Trp catabolism a few weeks after treatment. To confirm the role of liver fibrosis in Trp catabolism after SVR and to avoid the potential impact of IFN treatment, further assessments of later post-SVR follow-up time points (eg, 12 or 18 months) will be helpful. Furthermore, to better define the role of liver syndromes on the Trp catabolism, assessment of nonresponders for the treatment could be of interest.

In conclusion, ART-treated HIV/HCV-coinfected patients exhibited an enhanced level of immunosuppressive Trp catabolism, associated with the consequence of HCV infection liver fibrosis, rather than HCV infection itself. SVR did not reestablish normal Trp catabolism suggesting that liver damage can persist even after HCV cure and continue to induce a systemic inflammation by increasing Trp catabolism at least in the short term.

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