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Association of Interleukin-15–Induced Peripheral Immune Activation with Hepatic Stellate Cell Activation in Persons Coinfected with Hepatitis C Virus and HIV

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Abstract

Hepatic stellate cells (HSCs) mediate hepatitis C virus (HCV)–related liver fibrosis, and increased HSC activation in human immunodeficiency virus (HIV)/HCV coinfection may be associated with accelerated fibrosis. We examined the level of HSC activation in HIV/HCV-coinfected and HCV-monoinfected subjects and its relationship to the level of activation and gene expression of peripheral immune cells in coinfected subjects. HSC activation levels positively correlated with peripheral $CD4^+$ and $CD8^+$ T cell immune activation and were associated with enhanced interleukin-15 (IL-15) gene expression, suggesting a pathogenic role for IL-15–driven immunomediated hepatic fibrosis. Future strategies that reduce immune activation and HSC activation may delay progression of liver fibrosis.

An estimated one-third of people infected with human immunodeficiency virus (HIV) are also infected with hepatitis C virus (HCV), with ~200,000 HIV/HCV-coinfected people in the United States alone [1]. Among HIV-infected persons, the introduction of antiretroviral therapy (ART) has resulted in an increased survival duration but a subsequently increased risk of developing HCV-related liver disease.

HIV/HCV coinfection is associated with a more rapid progression of liver fibrosis than is observed in chronic HCV infection alone [2,3,4]. The hepatic stellate cell (HSC), a vitamin A–storing liver mesenchymal cell, has recently emerged as the most important fibrogenic cell in the liver related to hepatitis C–mediated fibrosis [5]. When activated by hepatic injury, HSCs undergo transformation into proliferative, fibrogenic, myofibroblast-like cells. After years of chronic liver inflammation, extracellular matrix deposited by HSCs accumulates and ultimately causes liver fibrosis, cirrhosis, and liver failure. The α isotype of actin (ASMA), a phenotypic marker of smooth muscle cells, is expressed by activated hepatic stellate cells [6]. Increases in

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ASMA have been shown to be correlated with progression of fibrosis to cirrhosis in HCV-infected patients [6]. An increased number of ASMA-positive hepatic stellate cells has also been described as an unfavorable event related to progression to cirrhosis [6]. However, the role of HSC activation has not yet been studied in HIV/HCV-coinfected subjects. In this study, we examined the relationship between HSC activation and activation markers and gene expression profiles in peripheral blood mononuclear cells (PBMCs) in HIV/HCV–coinfected subjects.

Subjects, materials, and methods

HIV/HCV-coinfected study subjects were recruited from clinical trials performed at the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH; Bethesda, MD), from 2001 through 2006. Forty-seven HIV-infected subjects received a weekly subcutaneous dose of peginterferon alfa-2b (1.5 μg/kg; Peg-Intron; Schering-Plough) or peginterferon alfa-2a (180 μg; Pegasys; Roche Laboratories) and daily oral ribavirin therapy (Rebetol [Schering-Plough]; 400 mg every morning and 600 mg every evening for persons weighing <75 kg and 600 mg twice daily for those weighing >75 kg) for 48 weeks and were followed up for 24 weeks after the end of treatment. Forty-seven HCV-monoinfected subjects were recruited from an ongoing HCV natural history study performed at the Department of Transfusion Medicine, Clinical Center, NIH, from 1990 through 2007. HCV-monoinfected subjects had never received treatment for HCV. All subjects signed an informed consent approved by the respective institutional review boards before enrollment in the study.

Liver biopsy specimens (length, 1.0–2.5 cm; diameter, 0.1 cm) were obtained from 47 monoinfected subjects ≤1 year before enrollment and from 30 coinfected subjects at the end of treatment. ASMA expression in HSCs was evaluated in fixed and paraffin-embedded liver biopsy tissue, using a monoclonal antibody in a standardized procedure with the avidin-biotin complex method [7,8]. The presence of ASMA immunoreactive cells was semiquantitatively scored using a modification of the Schmitt-Graeff method while blinded to infection status [9]. A score of 0 was given for <1% ASMA staining, 1 for 1%–10%, 2 for 10%–30%, 3 for 30%–70%, and 4 for >70%. ASMA-immunoreactive HSCs were scored separately in no fewer than 5 liver acini in each sample in periportal, intermediate, and perivenular zones.

Liver chemistry analysis and quantitative HCV RNA measurement (Versant HCV RNA 3.0 assay; Bayer Diagnostics) were performed on all subjects at the time of liver biopsy; immune markers on peripheral blood mononuclear cells and safety laboratory tests were performed before initiation of the treatment regimens and during each study visit.

DNA microarray analysis (Affymetrix HG-U133A) was performed on PBMCs, as previously described [10]. One-way analysis of variance (Partek Genomics Suite) was performed based on assigning subjects into 3 groups, stratified by difference in ASMA level (i.e., increase, decrease, and no change), followed by post hoc analysis based on the 3 possible pair-wise comparisons between the 3 subject groups. P values of <.05 and $\log_2$ mean fold differences (MFDs) of >0.38 or <−0.38 between the groups at baseline and at the end of treatment were separately considered to indicate statistical significance. The genes associated with ASMA increase or ASMA decrease ($P < .05$; absolute fold change, >1.3) were further characterized using the functional annotation tool DAVID.

HIV/HCV-coinfected subjects were matched to HCV-monoinfected subjects by Ishak fibrosis stage and then by age, sex, and race. Subjects were considered to be a match if their ages were within 5 years. Only pretreatment data for coinfected subjects were used in the matching analysis. Analyses were performed with standard statistical packages (SPSS, version 15.0 for Windows [SPSS], and SAS, version 8.2 [SAS Institute]). For descriptive statistics, continuous

variables were reported as the mean. For comparisons between 2 groups, continuous variables were compared using the Student *t* test. For parametric and nonparametric correlations between continuous variables, the Pearson correlation and Spearman's rho were used, respectively. For all analyses, a *P* value of <.05 was considered significant.

Results

Among 47 HIV/HCV-coinfected subjects enrolled, 41 (87%) were male, 26 (55%) were African American, and the mean age at the time of liver biopsy was 45.7 years. The following data were collected prior to initiation of HCV treatment. The mean HCV load was 3.12×10^6 copies/mL, and 40 subjects (85%) had HCV genotype 1 infection. The mean $CD4^+$ T cell count was 592 cells/μL, and no one had a count of <200 cells/μL. HIV levels were <50 copies/mL in 33 (70%) subjects. Mild-to-moderate hepatic fibrosis (Ishak stage 0–2) was found in 32 (68%), and bridging fibrosis or cirrhosis (Ishak stage 3–6) was found in 15 (32%). Among 47 pairs of matched coinfected and monoinfected subjects, 47 (100%) were matched by Ishak stage, 39 (83%) by age, 42 (89%) by sex, and 30 (64%) by race. HSC activation was not significantly greater in coinfected subjects, compared with monoinfected subjects (ASMA 10.9% vs. 8.0%; $P = .38$) but showed a larger nonsignificant difference in 20 subjects whose $CD4^+$ T cell counts were <500 cells/μL (ASMA 12.6% vs. 6.4%; $P = .22$). There were no significant differences in hepatitis activity index, alanine aminotransferase level, aspartate aminotransferase level, or HCV load between coinfected and HCV-monoinfected matched pairs. When we compared markers on peripheral immune cells to the degree of HSC activation among HIV/HCV-coinfected subjects, CD4 and CD8, CD38 and DR, and CD19 (i.e., B cells) correlated significantly with the change in ASMA level (table 1). A stronger correlation between $CD4^+$ T cell and B cell activation and change in ASMA level was observed among 20 coinfected subjects whose $CD4^+$ T cell counts were <500 cells/ μL (table 2).

To understand the relationship between peripheral gene expression profiles and HSC activation, we performed DNA microarray analysis on all subjects who underwent liver biopsy (figure 1). A total of 100 genes were identified that were differentially regulated among the 3 groups of subjects stratified by change in ASMA level (i.e., increase, decrease, or no change). Of these, 29 genes in pretreatment PBMC samples and 71 genes in posttreatment PBMC samples were differentially expressed. The gene encoding IL-15 was the only one that was expressed at levels that were consistently significant in the pair-wise comparison between the group in which ASMA increased and the group in which ASMA decreased between baseline and after treatment ($P < .02$) (figure 1). As shown in figure 2 and figure 3, expression of the gene encoding IL-15 (as measured by microarray analysis and real-time PCR) was markedly up-regulated at baseline and after HCV treatment in the group of subjects who had an increase in HSC activation and peripheral immune activation. Similarly, subjects who experienced a decrease in HSC and peripheral immune activation had lower baseline IL-15 expression, which further decreased with HCV treatment. Subjects who had no change in HSC activation with HCV treatment had low baseline IL-15 expression, which returned to normal after treatment. Following HCV treatment, IL-15 expression positively correlated with HSC activation (Pearson $r = 0.476$; $P = .025$).

Discussion

Our study demonstrates a direct correlation between the level of peripheral T and B cell immune activation and HSC activation in HIV/HCV-coinfected subjects. Our study also demonstrates a strong association between IL-15 gene expression in PBMCs and HSC activation. Accelerated progression of hepatic fibrosis is known to occur in HIV/HCV-coinfected individuals. However, the mechanism(s) responsible for more-rapid progression has not been described. Persistent immune activation is a well-described phenomenon in HIV disease and

may be increased in HIV/HCV coinfection [11,12]. Activation of HSC is associated with collagen deposition and hepatic fibrogenesis. Hence, it is plausible that increased IL-15–driven immune activation in HIV/HCV coinfection could drive HSC activation and accelerate hepatic fibrosis; this effect would be enhanced when $CD4^+$ cell counts are low and immune activation is most pronounced.

Levels of IL-15 gene expression were consistently associated with HSC activation. In subjects who had diminished or stable HSC activation as determined by the ASMA scores after completion of treatment with pegylated interferon alfa-2 and ribavirin, a similar trend in IL-15 gene expression was observed. These results suggest a role for IL-15 in inducing both peripheral and hepatic inflammatory response in HIV/HCV-coinfected subjects.

IL-15 is important for the maintenance of long-lasting, high-avidity T cell responses to invading pathogens, and it achieves this by supporting the survival of $CD8^+$ memory T cells, often quantified by high CD44 expression [13]. These functions include stimulating the proliferation of activated $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+$, and $CD8^+$ T cells, as seen with our subjects. Relative expression of the CD44 molecule subdivides $CD8^+$T cells into the following distinct subsets: those with a naive phenotype ($CD44^{lo/int}$) and those with a memory phenotype ($CD44^{hi}$) [13]. Interestingly, IL-15 facilitates the induction, activation, and proliferation of cytotoxic T lymphocytes and promotes the maintenance of $CD8^+CD44^{hi}$ memory T cells while inhibiting apoptosis. Future studies are needed to determine whether IL-15 is capable of driving HCV-specific cytotoxic T lymphocytes, which may be primarily responsible for HSC activation and fibrogenesis. Our attempts to quantify IL-15 in the serum of these subjects were not successful, mainly because IL-15 is secreted only in small quantities and is principally membrane bound. It induces signaling in the context of cell–cell contact; at the immunological synapse, signals from the IL-15R are delivered together with costimulatory signals that modify the response of the cell. It should also be noted that IL-15 is a proinflammatory cytokine, often described as being at the apex of a proinflammatory cytokine cascade [13]. It has been suggested that IL-15 expression might precede expression of tumor necrosis factor and the downstream cytokines IL-1, IL-6, and granulocyte/macrophage colony-stimulating factor, as well as the proinflammatory chemokines CCL- 3 (MIP-1α), CCL4 (MIP-1β), and CXCL8 (IL-8). As suggested by our data, IL-15 might contribute to ongoing hepatic cell damage by facilitating the maintenance of $CD8^+$ memory T cell survival, including that of self-reactive or HCV-reactive memory T cells. Dysregulated expression of IL-15 has been reported in patients with a range of autoimmune inflammatory diseases, including rheumatoid arthritis, multiple sclerosis, ulcerative colitis, refractory celiac disease, psoriasis, sarcoidosis, and hepatitis C, as well as diseases associated with the retrovirus HTLV-1 [13].

Our study failed to show a significant difference in the levels of HSC activation between HCV-monoinfected andHIV/HCV-coinfected subjects. One reason could be that the mean $CD4^+$ T cell counts in our subjects were generally in the normal range, exceeding 500 cells/μL, whereas past studies have found that more-pronounced immunosuppression (<500 cells/μL and particularly ≤200 cells/μL) was associated with increased fibrosis progression [4,14]. Additionally, 70% of our coinfected subjects had fully suppressed HIV loads with ART; prior studies have shown that HIV/HCV-coinfected individuals with suppressed HIV loads have rates of fibrosis progression similar to those of HCV-monoinfected individuals [14]. These factors may account, in part, for our failure to observe significant differences in HSC activation between matched coinfected and monoinfected individuals. However, consistent with our hypothesis, persons whose $CD4^+$ T cell counts were <500 cells/μL had the greatest degree of immune cell and HSC activation, but because this number of subjects was small, statistical significance was not demonstrated. Future studies will assess immune and HSC activation in subjects with unsuppressed HIV loads and/or more marked immunosuppression.

Acknowledgments

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References

1. Sulkowski MS, Thomas DL. Hepatitis C in the HIV-infected person. Ann Intern Med 2003;138:197–207. [PubMed: 12558359]
2. Thomas DL, Shih JW, Alter HJ, et al. Effect of human immunodeficiency virus on hepatitis C virus infection among injecting drug users. J Infect Dis 1996;174:690–695. [PubMed: 8843204]
3. Soto B, Sanchez-Quijano A, Rodrigo L, et al. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. J Hepatol 1997;26:1–5. [PubMed: 9147999]
4. Benhamou Y, Bochet M, Di Martino V, et al. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. Hepatology 1999;30:1054–1058. [PubMed: 10498659]
5. Friedman SL. Mechanisms of disease: mechanisms of hepatic fibrosis and therapeutic implications. Nat Clin Pract Gastroenterol Hepa tol 2004;1:98–105.
6. Guido M, Rugge M, Leandro G, et al. Hepatic stellate cell immuno-detection and cirrhotic evolution of viral hepatitis in liver allografts. Hepatology 1997;26:310–314. [PubMed: 9252139]
7. Skalli O, Ropraz P, Trzeciak A, et al. A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 1986;103:2787–2796. [PubMed: 3539945]
8. Gugliotta P, Sapino A, Macri L, et al. Specific demonstration of myo-epithelial cells by anti-alpha smooth muscle actin antibody. J Histochem Cytochem 1988;36:659–663. [PubMed: 3367051]
9. Schmitt-Graeff A, Krueger S, Bochard F, et al. Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 1991;138:1233–1242. [PubMed: 2024709]
10. Lempicki RA, Polis MA, Yang J, et al. Gene expression profiles in hepatitis C virus (HCV) and HIV coinfection: Class prediction analyses before treatment predict the outcome of anti-HCV therapy among HIV-coinfected persons. J Infect Dis 2006;193:1172–1177. [PubMed: 16544259]
11. Hunt PW, Martin JN, Sinclair E, et al. T cell activation is associated with lower $CD4^+$ T cell gains in human immunodeficiency virus–infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 2003;187:1534–1543. [PubMed: 12721933]
12. Kovacs A, Al-Harthi L, Christensen S, et al. $CD8^+$ T cell activation in women coinfected with human immunodeficiency virus type 1 and hepatitis C virus. J Infect Dis 2008;197:1402–1407. [PubMed: 18444798]
13. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nat Rev Immunol 2006;6:595–601. [PubMed: 16868550]
14. Brau N, Salvatore M, Rios-Bedoya CF, et al. Slower fibrosis progression in HIV/HCV-coinfected patients with successful HIV suppression using antiretroviral therapy. J Hepatol 2006;44:47–55. [PubMed: 16182404]

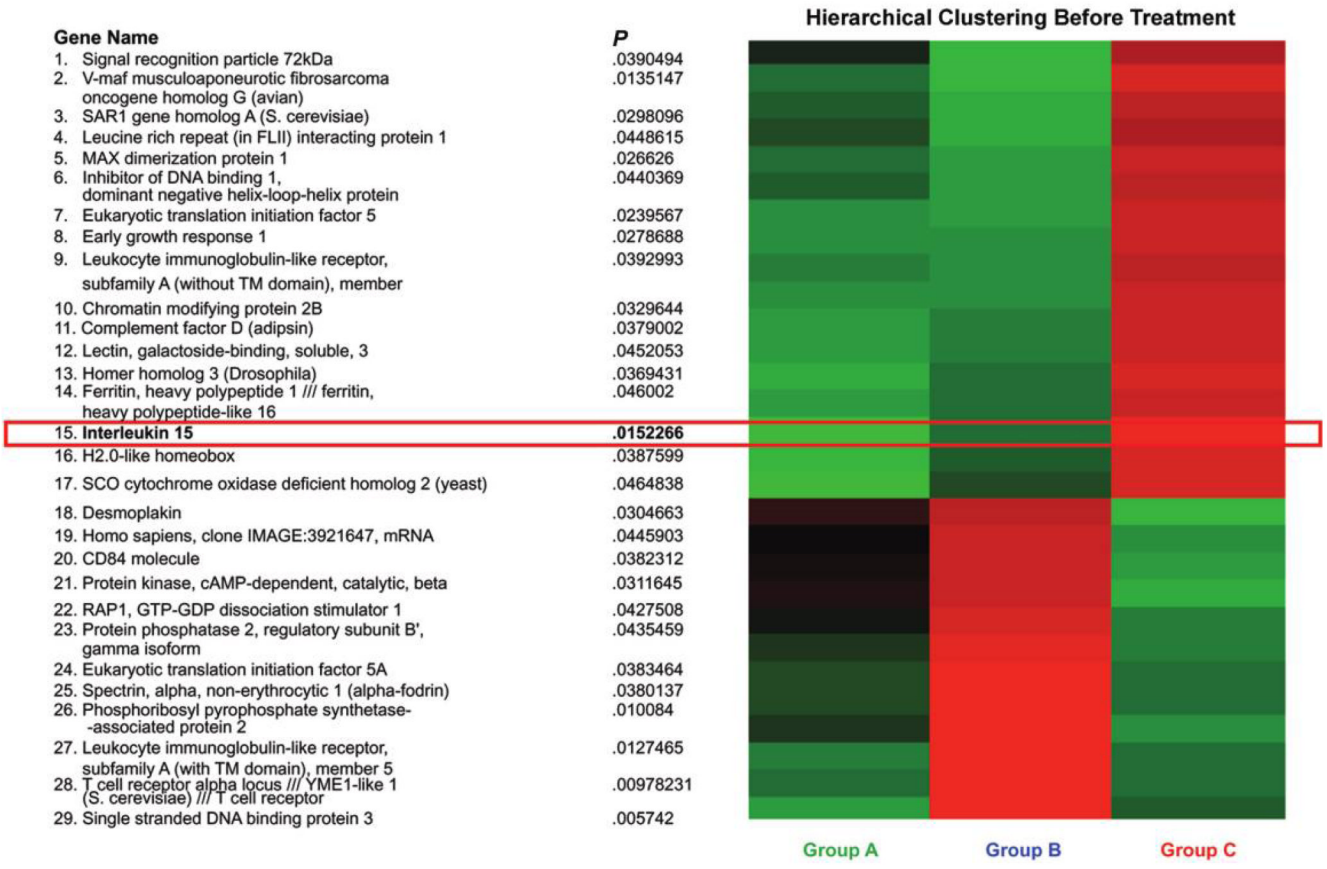

Figure 1.

At baseline, these genes were significantly associated with an increase (group C) or decrease (group B) in α-smooth muscle actin (ASMA), a marker of hepatic stellate cell (HSC) activation ($P < .05$; absolute mean fold difference, >1.3). The gene encoding interleukin-15 (IL-15) was the only gene that presented consistently significant values in the pair-wise comparison between groups C and B, before treatment ($n = 29$ signature genes) and after treatment ($n = 71$ signature genes; data not shown). Group A consisted of subjects who experienced no changes in the ASMA score after therapy. ASMA was not associated with treatment response ($P = .702$).

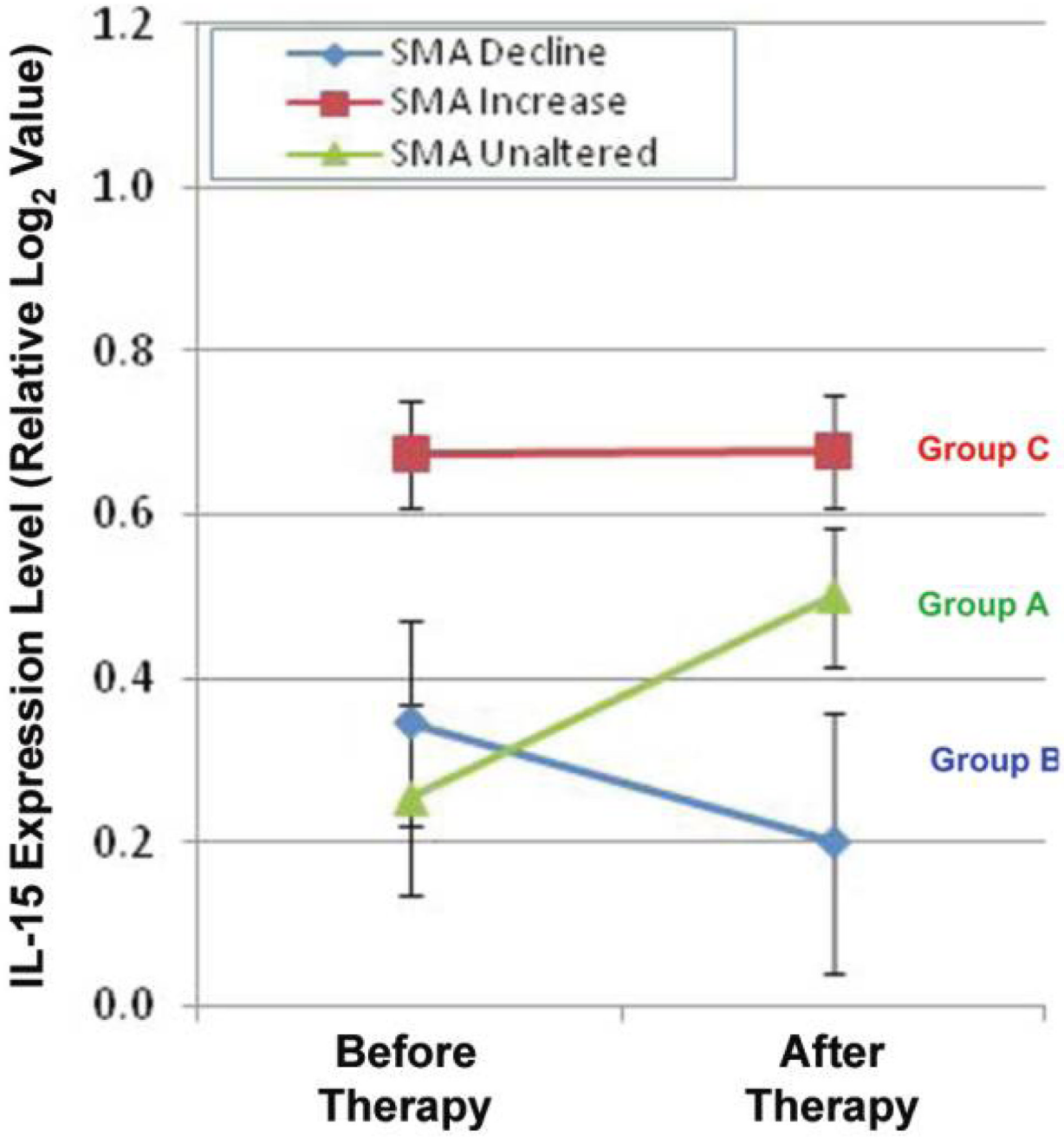

Figure 2.

Increased levels of expression of the gene encoding interleukin-15 (IL-15) were significantly associated with increased α-smooth muscle actin (ASMA) levels before and after interferon-α/ribavirin therapy in microarray analysis (group C; $P < .01$). These results were validated by real-time polymerase chain reaction (PCR), reflecting significant differences in the IL-15 gene expression in the pair-wise comparisons between group B and C and between groups A and C before and after interferon-α/ribavirin therapy ($P < .01$). Although real-time PCR revealed that IL-15 expression appeared to increase for all groups (A, B, and C) after therapy, these changes were not significant. Results are for 22 liver biopsy specimens obtained before and 22 obtained after treatment that were included in microarray analysis. The mean fold difference (MFD)

was calculated for each gene at 2 different time points (i.e., before and after therapy) as follows: for microarray analysis and real-time PCR, ($\log_2$) MFD = [mean expression ($\log_2$) in group C] – [mean expression ($\log_2$) in group B]; for real-time PCR, ($\log_2$) MFD = [mean expression ($\log_2$) in group C] – [mean expression ($\log_2$) in group A. *Cutoff for statistical significance: $P < .05$ and ($\log_2$) MFD >0.38 or <−0.38 (which is equal to an absolute MFD of >1.3 or < −1.3). The mean fold change (MFC) was calculated for each gene within each group (A, B, and C) at 2 different time points (i.e., before and after therapy) as follows: for microarray analysis and real-time PCR, ($\log_2$) MFC = [mean expression ($\log_2$) after therapy] – [mean expression ($\log_2$) before therapy]. **Cutoff for statistical significance: $P < .05$ and ($\log_2$) MFC >0.38 or <−0.38 (which is equal to an absolute MFC of >1.3 or <−1.3).

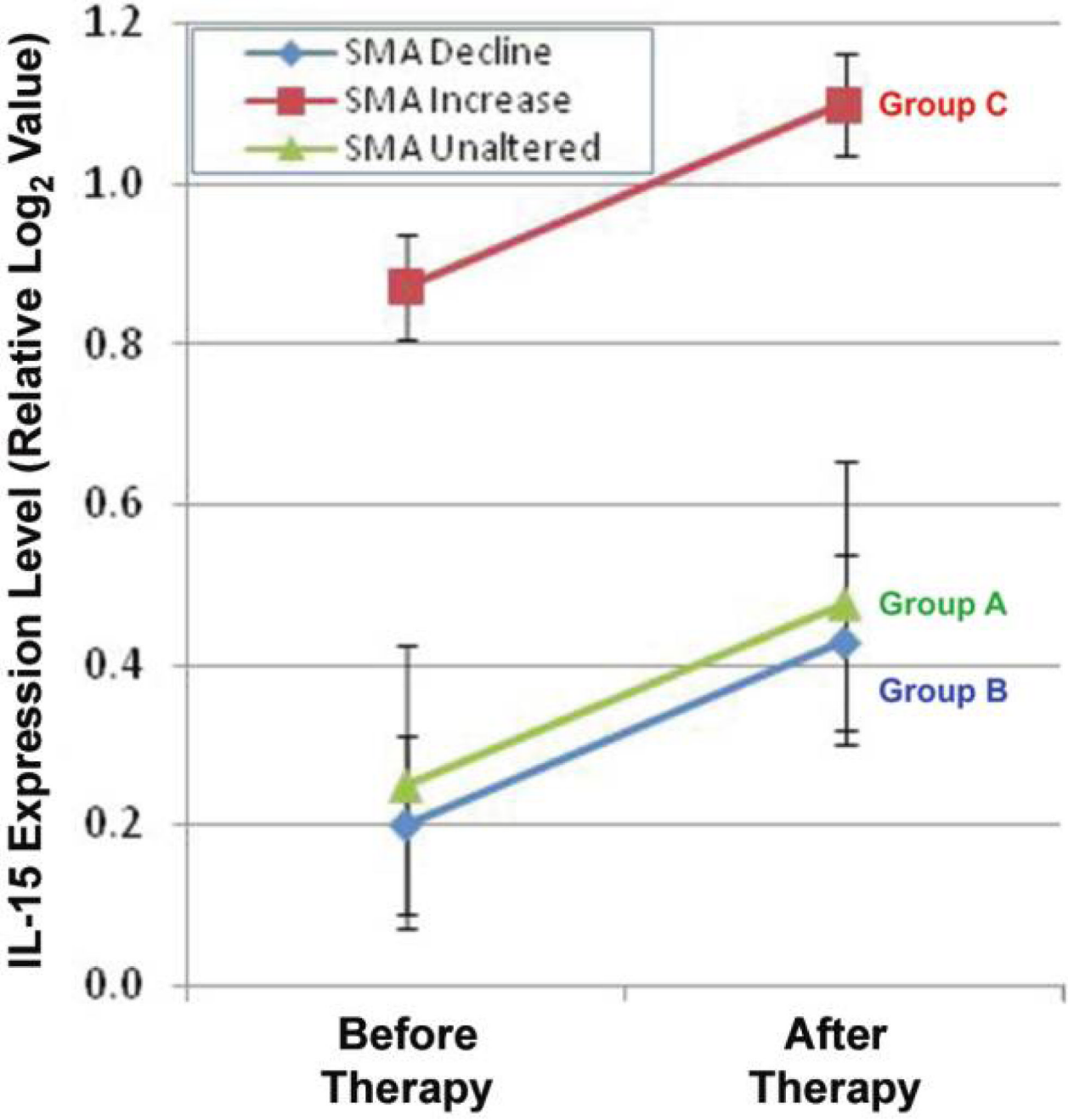

Figure 3.

Total RNA was isolated from peripheral blood mononuclear cells (PBMCs), and 150 ng was used for reverse transcription (RT) analysis, using the manufacturer's recommend protocol (ABI reverse transcription kit). One-fifteenth of the RT reaction mix was used for real-time polymerase chain reaction (PCR; in triplicate), using ABI′ Taqman gene expression master mix and the ABI 7900HT fast real-time PCR system. IL-15 and GAPDH primers and probes were ordered through ABI's premade Taqman gene expression assays system: IL-15 probe set A (ABI assay ID: HS9999039_m1) specific for an IL-15 coding exon; IL-15 probe set B (ABI assay ID: Hs01003716_m1) specific for the IL-15 3′ UTR target by Affymetrix's U133A IL-15

probe set 205992_s_at; and GAPDH (ABI assay ID: Hs01003716_m1). IL-15 expression data were normalized to GAPDH expression levels and expressed as relative $\log_2$ values.

Table 1

Correlation of T and B Cell Activation with Hepatic Stellate Cell Activation

Variable	ASMA, %	
	Correlation coefficient	*P*
Cell activation marker, %		
CD4CD38	0.311[a]	.034
CD4DR	0.350[a]	.016
CD8CD38	0.289[b]	.049
CD8DR	0.317[b]	.030
Cell activation marker, cells/μL		
CD4DR	0.343[b]	.018
CD8DR	0.334[b]	.022
CD19	0.289[a]	.049

NOTE. Data are for 47 subjects coinfected with human immunodeficiency virus and hepatitis C virus. ASMA, α-smooth muscle actin.

[a] Pearson *r*.

[b] Spearman's rho.

Table 2

Correlation of T and B Cell Activation with Hepatic Stellate Cell Activation

Variable	ASMA, %	
	Correlation coefficient	*P*
Cell activation marker, %		
CD4	0.450[a]	.047
CD38	0.495[b]	.026
CD4DR	0.457[b]	.043
Cell activation marker, cells/μL		
CD4	0.525[a]	.012
CD38	0.452[b]	.035
CD4DR	0.511[b]	.015
CD19	0.476[a]	.034

NOTE. Data are for 20 subjects coinfected with human immunodeficiency virus and hepatitis C virus for whom the $CD4^+$ cell count was <500 cells/μL. CD8 activation markers did not correlate with HSC activation when the $CD4^+$ cell count was <500 cells/μL. ASMA, α-smooth muscle actin.

[a] Pearson *r*.

[b] Spearman's rho.