Interleukin 2 Treatment Does Not Modify Hepatitis B or C Replication in Human Immunodeficiency Virus–Infected Patients: Results From a Randomized Control Trial

To the Editor:

Because of common routes of transmission, human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections are often found simultaneously in the same patient. Clinical consequences of such coinfection may lead rapidly to the development of severe liver-related complications.[1] We investigated the effects of interleukin-2 (IL-2) treatment in HIV patients coinfected with HBV, HCV, or both hepatitis viruses. Seventy-two HIV-infected patients were enrolled in a 6-month randomized controlled study (ANRS 082, ILSTIM) comparing subcutaneous injection of IL-2 (4.5 million international units [IU], twice daily, 5 days a week, every 6 weeks) in addition to their prior highly active antiretroviral treatment (HAART) (IL-2 group) versus HAART alone (control group).[2] Main criteria for inclusion were a controlled HIV infection under HAART containing a protease inhibitor for at least 6 months, with HIV viral loads below 1,000 copies/mL for at least 3 months, and CD4 cell counts between 25 and 200/mm^3 since the beginning of the protease inhibitor treatment. All patients who completed their treatment (n = 69) were tested for the presence of hepatitis B surface antigen and specific HCV antibodies (Axsym, Abbott, France). Viremia for each virus was determined at the time of inclusion and after IL-2 treatment completion (24 weeks). Nine patients (13%) tested positive for HBV, 12 (17.4%) for HCV, and 3 (4.3%) patients were HBV and HCV coinfected (Table 1). At the time of inclusion, HCV-positive patients had significantly higher alanine transaminase (ALT) levels than HCV-negative patients (median 76.5 vs. 24 IU/L, $P = .0005$), whereas no ALT difference was seen for HBV-infected patients. During IL-2 treatment, no significant change in ALT levels was observed in HBV-coinfected patients; by contrast, an important but not statistically significant drop ($P = .089$) was noticed for HCV-infected subjects. Interestingly, we did not notice any significant change in HBV or HCV viral load under IL-2 treatment compared with the control group.

The immunologic component in chronic viral hepatitis is not fully understood and seems to involve a balance between the host immune response and the virus itself. Thus, because of the pleiotropic effects of IL-2, one may speculate that an IL-2–induced shift towards a more potent immune response could help to clear viral infections. Very few trials have studied the effects of IL-2 on viral hepatitis, and comparison of these trials are made difficult because of the different IL-2 dosages and schedules used. However, 3 recent reports on a limited number of HIV-coinfected patients have looked at the effects of IL-2 on hepatitis virus replication.[3-5] While Uberti-Foppa et al. found a significant decrease in HCV viral loads and Gianotti et al. described clearance of hepatitis B surface antigen in a single patient, our data as well as those from Hengge et al. do not support any effect of IL-2 treatment on either HCV or HBV replication.

In conclusion, despite a significant increase in CD4 cell count in the IL-2–treated group compared with the control group patients (median increase in CD4: 65 vs. 18/mm^3, $P < 10^{-6}$), IL-2 treatment did not modify hepatitis viruses replication.[2] The decrease in transaminase levels in HCV-infected subjects may indicate that such treatment could affect liver disease progression; however, a more careful histologic and biochemical monitoring would be necessary to further address this question.

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Table 1. Main Biological Parameters (median, range) at Inclusion and 24 Weeks After IL-2 Treatment

	HBV Positive		HCV Positive		HBV and HCV Positive	
	Control	IL-2	Control	IL-2	Control	IL-2
n (%)	3	6	7	5	2	1
HBV viral load* at inclusion	Negative	5 (2.9-8.0)	NA	NA	Negative	Negative
HBV viral load* at week 24	Negative	4.9 (2.6-8.6)	NA	NA	Negative	Negative
HCV viral load* at inclusion	NA	NA	7.3 (6.6-8.0)	7.3 (6-8.3)	5.4/Negative	Negative
HCV viral load* at week 24	NA	NA	7.4 (6.8-7.9)	7.3 (6.4-8.4)	7.6/Negative	NAV
ALT† level at inclusion (IU/L)	14 (8-23)	33.5 (12-112)	54 (8-100)	115 (29-154)	78/125	25
ALT† level at Week 24 (IU/L)	11 (7-15)	35 (8-56)	49 (16-81)	45.5 (5-80)	81/115	12
CD4 count at inclusion (cells/mm^3)	108 (93-110)	163 (97-195)	157 (63-190)	161 (106-187)	174/124	94
CD4 count at week 24 (cells/mm^3)	127 (102-178)	251 (180-322)	160 (84-198)	203 (190-222)	281/146	208

Abbreviations: NA, not applicable; NAV, not available.
*Viral load medians (range) are expressed as Log10 copies/mL for HBV (Monitor-ROCHE) and as Log10 Equivalent genome/mL for HCV (bDNA2.0-BAYER).
†Normal value for ALT is below 35 IU/L.

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The 4,977–Base Pair Common Deletion of Mitochondrial DNA Is Not Associated With Steatosis in Chronic Hepatitis C Patients

To the Editor:

Mitochondrial DNA (mtDNA) deletions have been detected in the liver of patients with alcohol-, drug-, and metabolic-related steatosis and may play a role in the pathogenesis of liver disease.[1] At the 51st Annual Meeting of the American Association for the Study of Liver Diseases,[2] a 4,977-bp deletion (common deletion) of mtDNA was reported to be associated with the steatosis observed in chronic hepatitis C. We assessed the prevalence of the mtDNA common deletion in the liver of chronic hepatitis C patients and compared it with the presence and severity of liver steatosis. Two groups of chronic hepatitis B patients and healthy adults served as controls.

A liver biopsy was obtained from 69 patients with histologically confirmed chronic hepatitis C. There were 46 men and 23 women. The median age was 46 years (range, 30-70). Eighteen patients had cirrhosis and 23 were infected with HCV genotype 3, which has been associated with liver steatosis.[3] All lacked factors (other than hepatitis C virus) associated with a fatty liver, *i.e.*, chronic alcohol consumption, a body mass index (weight [kg]/height^2 [m^2]) greater than 26, decompensated diabetes, or the administration of hepatotoxic drugs. Macrovesicular steatosis was absent or minimal (<1% of hepatocytes) in 36 patients, mild (<30% hepatocytes) in 21, moderate (between 30% and 60% of hepatocytes) in 7, and severe (>60% of hepatocytes) in 5. Controls included 15 liver biopsy specimens from chronic hepatitis B patients and 8 normal adult livers taken at surgery (cholecystectomy or resection of liver metastasis of extrahepatic malignancies). None of the controls had steatosis.

A portion of each liver sample, adjacent to that being histologically evaluated, was snap-frozen for total liver DNA extraction.[3] To confirm the quality and quantity of extracted DNA, a first polymerase chain reaction (PCR) was performed with primers C1 (nt 9911-9932) and C2 (nt 11873-11851) (Fig. 1A).[4,5] To discriminate between deleted and undeleted mtDNA, we used a combination of PCR strategies. Two couples of primers, A-A1 and B-B1[5] or A-A1 and MT1-MT3[6] were chosen to perform a nested PCR (Fig. 1A). Primers flanked the 13-bp direct repeat associated with the mtDNA common deletion. Long Template Taq polymerase was purchased from Roche (Basel, Switzerland). The 6-kb product of undeleted mtDNA was expected after 3 minutes of

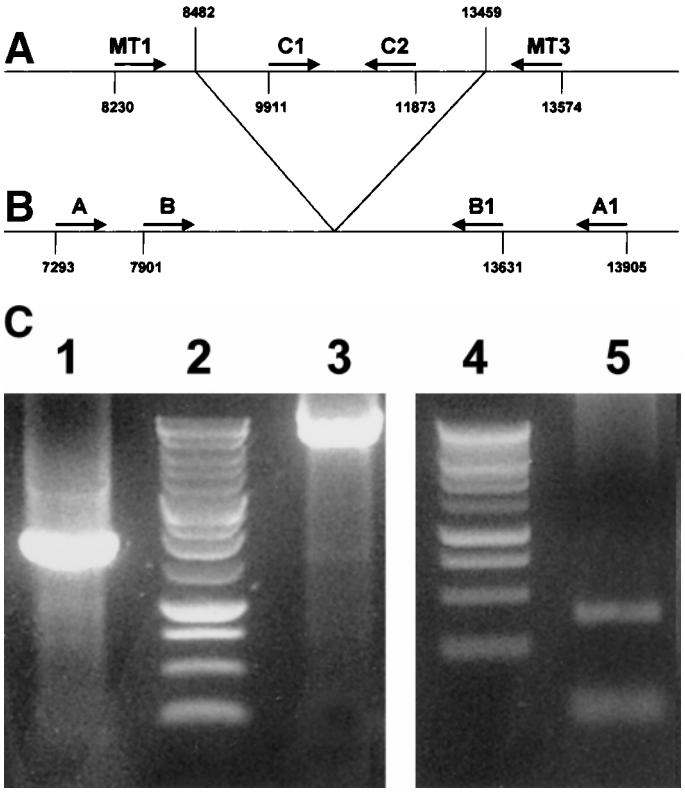

Fig. 1. (A and B) Map showing the position of the different primers used in the present work with respect to the mtDNA common deletion, located between base positions 8482 and 13459. Wild-type (A) and 4977-bp deleted form (B) of mtDNA. The circular mtDNA molecule is here represented as linearized for reasons of simplicity. (C) PCR products from total liver DNA using mtDNA-specific primers. Lanes 2 and 4, molecular size markers (1 kb DNA ladder, Promega, Madison, WI). Wild-type, undeleted mtDNA give rise to 2-kb (lane 1) and 6-kb (lane 3) PCR products using, respectively, primers C1-C2 and B-B1 (the latter ones with a prolonged elongation time; please refer to text for explanations). When the mtDNA presents the common deletion, primers MT1-MT3 yield a 360-bp product using a short elongation time (lane 5).

extension at 72°C for 30 cycles in both rounds of PCR. To selectively amplify the deleted form we used a shorter time of elongation (30 seconds at 72°C for 30 cycles).[6] The two PCR procedures yielded a product, respectively, of 770 or 360 nt, depending on the use of primers B-B1 or MT1-MT3 during the nested PCR.

PCR product(s) having a size compatible with the mtDNA common deletion were detected in 62 of 69 (91%) chronic hepatitis C patients (30 with steatosis) and in all controls (Fig. 1b). The mean age of the 62 chronic hepatitis C patients with the deletion was comparable with that of patients without (50 ± 7.3 years vs. 47 ± 9.5 years, $P = .32$). The presence of the common deletion was not associated with gender, HCV genotype, staging of liver disease, or presence and severity of steatosis.

The high prevalence of the 4,977-bp mtDNA common deletion found in our patients confirms its frequent detection in various tissues from individuals over 20 years of age.[7] However, we failed to associate the occurrence of the mutation with the presence of steatosis. The variable clinical presentation observed with this mtDNA mutation may depend on the number of mutated mtDNA molecules per cell and on its energy requirements.[8] The random segregation of mitochondria at the time of cell division results in an mtDNA heteroplasmy. Intracellular mixtures of mtDNA characterized by a high ratio of deleted versus wild-type mtDNA are likely to bring about a decline of cellular energy capacities. Thus, heteroplasmy and the tissue specific energy threshold are important disease determinants and should be taken into account when considering the possible pathogenicity of an mtDNA mutation. Whether the presence of liver steatosis depends on the number of mutated mtDNA molecules per hepatocyte, rather than on their occurrence, remains to be established by appropriate quantitative assays.[8] Thus, the lack of association with steatosis may be caused by the failure to identify different ratios of deleted versus wild-type mtDNA using a qualitative PCR.

We conclude that, based on a sensitive qualitative PCR screening, the common deletion of mtDNA is highly prevalent in the liver of patients with hepatitis C virus, independent of histology. In particular, we found no association with the occurrence and severity of liver steatosis.

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HDL Receptor SR-BI and Cholesterol Gallstones

To the Editor:

We read with great interest the recent report of Fuchs et al.[1] on the up-regulation of hepatic scavenger receptor class B type I (SR-BI) and caveolin-1 expressions associated with biliary cholesterol hypersecretion and gallstone formation in gallstone-susceptible C57L mice compared with resistant AKR mice. While SR-BI is a functionally relevant HDL receptor for reverse cholesterol transport,[2] caveolin-1 appears to be important for intracellular cholesterol trafficking.[3] Thus, the study of Fuchs et al.[1] is consistent with a potential role of these cholesterol transport proteins in the molecular pathogenesis of murine cholesterol gallstone formation.

However, we would like to point out that similar studies performed independently by our two groups do not agree with or support the conclusions obtained by Fuchs et al.[1] First, we observed that on chow, there were no differences in hepatic SR-BI protein expression between C57L and AKR mice. Furthermore, feeding a lithogenic diet (1% cholesterol, 0.5% cholic acid, and 15% dietary fat) did not induce a significant alteration in hepatic SR-BI levels in these mice (Rigotti et al. and Wang et al., unpublished observations).

Second, using gallstone-susceptible C57BL/6 mice,[4] we studied hepatic SR-BI and caveolin-1 protein expression during the feeding of a lithogenic diet from Harlan Teklad. Amigo et al.[5] found that biliary cholesterol hypersecretion during gallstone formation in these mice was associated with a decrease in plasma HDL cholesterol. However, these alterations in HDL cholesterol concentrations and biliary cholesterol secretion rates induced by the lithogenic diet in C57BL/6 mice did not correlate with any changes in hepatic SR-BI protein expression.[6] Furthermore, we did not detect significant up-regulation of caveolin-1 protein levels in the liver of C57BL/6 mice fed the lithogenic diet compared with chow (Miquel et al., unpublished observations).

Third, one of us has addressed more directly the potential relevance of SR-BI expression for gallstone formation in mice during feeding of a lithogenic diet. Wang et al.[7] studied SR-BI att mice with a partial deficiency (~50%) of hepatic SR-BI expression induced by a dysfunctional mutation in the *Sr-bi* promoter. This study found that decreased SR-BI expression in gallstone-susceptible SR-BI att mice reduced biliary cholesterol secretion by 37% on chow and 10% on the lithogenic diet and modestly, but not significantly, decreased susceptibility to cholesterol gallstone formation. Mardones et al.[6] have also found that knockout of SR-BI gene decreased biliary cholesterol secretion by ~55% in chow-fed mice. Taken together, these results[6,7] strongly suggest that on chow, SR-BI is a major receptor regulating biliary cholesterol concentrations, however, it is much less relevant for controlling biliary cholesterol hypersecretion derived from intestinal chylomicrons during murine cholesterol gallstone formation on a lithogenic diet.[5,7]

Despite the discrepancy between our results and the data of Fuchs et al.,[1] SR-BI is still likely to be a candidate gene to be involved in gallstone formation due to increased HDL-mediated reverse cholesterol transport. We await further studies that support this hypothesis. If so, SR-BI may be an interesting drug target for prevention and treatment of this common disease afflicting western countries.

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Reply:

We appreciate the comments of Rigotti et al. on our recently published report[1] on the hepatic up-regulation of SR-BI during cholesterol gallstone formation in C57L but not AKR mice. Despite interindividual variations in hepatic SR-BI expression, we measured increased SR-BI protein levels in all C57L mice fed the lithogenic diet. Contrary to our findings and employing AKR and C57L mouse strains, their preliminary experiments did not show significant alterations in the expressions of SR-BI protein in liver. It is difficult to comment on these discrepancies, especially because we do not know whether experimental or methodological differences exist between our investigations and those performed in the laboratories of Drs. Rigotti and Wang.

Earlier studies using inbred mice[2-5] showed variations in gallstone susceptibility in response to a lithogenic diet. Whereas gallstone prevalence was high in C57L/J mice, the closely related but not genetically identical C57BL/6 strain was notable for an intermediate prevalence. Subsequent genetic studies showed that this most likely is related to different *Lith* genes in these 2 inbred mouse strains.[6] Thus, it is not surprising that C57BL/6 mice apparently do not up-regulate hepatic protein expression of SR-BI and caveolin-1 (Miquel et al., unpublished observations). Indeed, our own preliminary results (Fuchs et al., unpublished observations) with 3-month-old male C57BL/6 mice also found no significant changes in SR-BI protein expression when the animals were challenged with a commercially obtained lithogenic diet from Harlan Teklad. Instead, these findings support a complex genetic regulation of HDL cholesterol metabolism,[7-9] which does not depend only on SR-BI expression.

The interesting preliminary findings of Wang et al.[10] obtained in mice with a partial deficiency of hepatic SR-BI protein expression do not necessarily contrast our finding of hepatic up-regulation of SR-BI during cholesterol gallstone formation. It is likely that the genetic manipulation in these mice induced the

expression of other genes involved in cholesterol and lipoprotein metabolism, which in part may compensate for the SR-BI deficiency. In addition it is important to mention that the SR-BI att mice are maintained in a mixed BALB/cByJ × 129 Sv strain background.[11] This may be important, too, because BALB mice are characterized by a low cholesterol gallstone prevalence when challenged with a lithogenic diet.[4]

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Promoter Polymorphism of the CD14 Endotoxin Receptor Gene and Primary Biliary Cirrhosis

To the Editor:

We have read with interest the recent work by Jarvelainen et al.[1] The investigators reported that the C(-159)T promoter polymorphism of the CD14 endotoxin receptor gene, which was found to confer increased expression of CD14,[2] may be associated with the risk and severity of alcohol-induced liver disease. Because the CD14 receptor is an important mediator for the activation of monocytes/macrophages by endotoxins, this finding lends support to the hypothesis that activation of Kupffer cells by gut-derived endotoxins plays a key role in promoting pathologic liver injury in alcohol-induced liver disease.

In general, one can speculate that bacterial endotoxins and liver-derived CD14-positive monocytes/macrophages may contribute more largely to the initiation and/or the progression of chronic liver diseases. In this view, several observations strongly suggest that enterobacterial antigens might play a crucial role in the pathogenesis of primary biliary cirrhosis (PBC). For example, it has been shown that serum antibodies against lipopolysaccharide (LPS), a component of gram-negative bacteria, and lipid A (a component of LPS), were more frequently found in patients with PBC than in controls.[3,4] Furthermore, unusual concentrations of endotoxins were detected in the liver, bile, and peripheral blood of patients with PBC,[5,6] and parenteral administration of LPS in mice was shown to induce portal inflammation, bile duct proliferation and biliary epithelial cell degeneration.[7] Finally, the CD14 receptor was detected on mononuclear cells around the damaged bile ducts in PBC.[8]

These observations prompted us to investigate whether the promoter polymorphism of the CD14 gene could contribute to the predisposition and the severity of PBC. For that purpose, we performed a polymerase chain reaction (PCR) amplification of the

Distributions of C(−159)T CD14 Genotypes and Alleles in Patients With PBC and Controls

	PBC Patients (n = 30)	Controls (n = 27)	*P*
CD14 genotypes			
C/C	5 (16.7%)	6 (22.2%)	
C/T	19 (63.3%)	15 (55.6%)	NS
T/T	6 (20.0%)	6 (22.2%)	
CD14 Alleles			
C	29 (48.3%)	27 (50.0%)	NS
T	31 (51.7%)	27 (50.0%)	

promoter of the CD14 gene on genomic DNA extracted from white blood cells of 30 women with PBC, as confirmed by positivity for antimitochondrial antibody and compatible liver histology, and 27 age- and sex-matched control subjects. The C(-159)T CD14 genotype was subsequently determined by restriction fragment-length polymorphism (RLFP) analysis with endonuclease *Hae*III, as previously described.[2] The CD14 allele and genotype distributions were compared between patients and controls using the χ^2 test. The relationships between the CD14 genotype and the characteristics of the patients were assessed using the Jonckheere-Terpstra test. There was no statistical difference between PBC patients and controls for the CD14 allele and genotype distributions (Table 1). There was neither significant correlation between the CD14 genotype and age, serum bilirubin level, activities of alkaline phosphatase (AP), alanine and aspartate aminotransferases (ALT and AST), serum albumin level, prothrombin time, platelet count, serum IgM level, and histologic stage, as determined at the time of diagnosis. Finally, the magnitude of changes in serum bilirubin, AP, AST, and ALT levels assessed after 4 years of ursodeoxycholic acid (UDCA) therapy did not differ between the three CD14 genotypes. We conclude that, unlike alcohol-induced liver disease, PBC does not show significant association with the C(-159)T promoter polymorphism of the CD14 receptor gene.

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