Intravenous drug abuse is the major cause of transmission of hepatitis C virus (HCV) in the western world, infecting 150 million adults worldwide. Throughout the last 20 years, it has become apparent that opiates have significant effects on immune function. In this, and a previous issue of *The American Journal of Pathology*, Li and colleagues and Wang and colleagues have demonstrated that morphine enhances HCV replication in HCV replicon-containing cells. Now they show that morphine withdrawal also paradoxically enhances the expression of HCV, albeit transiently. These results may have major implications for treating patients who are currently either abusing drugs or receiving opioid substitutes such as methadone while infected with HCV because these opiates may impair clearance of virus from the body.

HCV is a positive strand RNA flavivirus that was first cloned in 1989. It has six distinct genotypes, with genotype 1 being the most common in the United States and Western Europe. The most common mode of transmission in developed countries is through shared needles by intravenous drug users (IVDUs). Once infected with HCV, an estimated 15 to 40% of infected individuals clear the virus spontaneously whereas the majority develops chronic HCV infection. HCV is now one of the most common causes of cirrhosis and hepatocellular carcinoma worldwide. There are an estimated 150 million cases of chronic hepatitis C worldwide, and perhaps 30% of those may eventually progress to cirrhosis or hepatocellular carcinoma. The rate of progression to cirrhosis is variable. Most studies estimate that it takes 10 to 30 years to develop cirrhosis in the majority of patients, with some not developing significant liver disease until 50 years after infection. Several factors may accelerate the progression of liver disease. The most notable of which are alcohol abuse, older age of acquisition, and HIV co-infection.

IVDUs require special access to care because they currently constitute the largest cohort of patients needing treatment in many western countries. There are numerous challenges in treating hepatitis C in this group. Psychiatric disease, nonadherence to treatment, risk of reinfection, social issues, and other co-morbid medical issues remain potential barriers to treatment. Thus, opiate-dependent IVDUs are candidates for treatment but require close supervision, special resources, and addiction medicine specialists to overcome the barriers to care. However, until recently there were no data on the effects of opiates on HCV replication or the development of liver injury and fibrosis, one of the earliest features of progression to cirrhosis.

**Connections between Opiates, HCV, and Liver Disease**

The study of HCV has been hampered by the lack of good animal models or cell culture systems. Recent genetic manipulations of the RNA of HCV have produced high levels of replication in cell lines derived from hepatocytes (eg, Huh7), offering a more feasible means of studying HCV replication. This system is termed the HCV replicon system and has been used successfully to study anti-HCV drugs such as interferon. Recently, full-length infectious HCV genomes that replicate in cell culture have been cloned.

In their first study Li and colleagues showed that the human hepatocellular carcinoma-derived cell lines Huh8, Huh7, and FCA-1 express opioid receptors by using reverse transcriptase-polymerase chain reaction. When transfected with the HCV replicon and exposed to low concentrations (0.1 nmol/L to 1 μmol/L) of morphine (the major metabolite of heroin), the level of HCV RNA increased threefold, but these effects diminished by 4 days. They also showed an association with increased expression of the NS5 protein, which plays a critical role in HCV replication. The effects of morphine on HCV replication were blocked by naltrexone, a pan-opioid antagonist, as well as by a- and b-opioid receptor antagonists. To investigate whether morphine activates nuclear factor (NF)-κB, a nuclear transcription factor that controls viral replication and cytokine production, they used Huh7 cells transfected with the plasmid pNFκB-A.
Luc, which contains the NF-κB promoter linked to the luciferase gene. They showed that morphine causes activation of NF-κB and that a NF-κB inhibitor (CAPE) blocks morphine-induced viral replication. The implication of this study is that IVDUs who are infected with HCV and who continue to inject heroin (diamorphine), either daily or intermittently, may be causing sudden surges of HCV replication. Although HCV viral load is a predictor of the response to combination therapy, it is unclear if fluctuations in viral load, perhaps due to concurrent opiate abuse, affect the therapeutic response to anti-viral therapy.

In a follow-up study appearing in the current issue of The American Journal of Pathology,6 the same laboratory paradoxically shows that after chronic morphine treatment (4 days) of HCV replicon-transfected cells, morphine withdrawal from the culture medium resulted in an upsurge of HCV replication of up to 2.5-fold by 48 hours. These effects, however, were only transient as HCV RNA levels returned to baseline by 72 hours. These changes in viral replication were also accompanied by changes in the expression of the NS5A protein. The upsurge in viral replication was associated with a marked decrease (~80% reduction) in endogenous interferon (IFN)-α synthesis by the HCV replicon cells due to suppressed activity of the IFN-α promoter and its activator interferon-regulatory factor-7 (IRF-7).

The data from both the current and previously published studies suggest that continued, intermittent use of opiates with and without acute withdrawal leads to a greater risk of increased HCV replication and, by inference, greater long-term liver injury and enhanced risk of developing cirrhosis and hepatocellular carcinoma. It is not generally appreciated by clinicians who treat long-term IVDUs that while the role of opiates on HCV and HIV replication have only been described relatively recently, it has been known for some time that opiates such as morphine have a marked effect on immune function. Receptors for morphine and the endogenous opioid peptide β-endorphin, a neuropeptide released from the anterior pituitary during stress, have been described for at least two cell types involved in cell-mediated immunity, namely T lymphocytes and mononuclear phagocytes. Peterson and colleagues8,9 showed nearly 20 years ago that morphine suppresses the production of IFN-γ by cultured human peripheral blood mononuclear cells and that this could be blocked by naltrexone, a nonspecific opioid receptor antagonist. Although these data demonstrate this to be a receptor-dependent process, it was also shown that inhibition of prostaglandin synthesis by indomethacin prevents opiate-induced reduction of IFN-γ synthesis and that superoxide dismutase or catalase could both completely or partially prevent morphine-induced reduction of IFN-γ synthesis.

The microbicidal function of mononuclear phagocytes depends on their respiratory burst, during which superoxide anion (O2−) is secreted to facilitate microbial killing. When added acutely, opiates such as morphine stimulate a respiratory burst in peripheral blood polymorphonuclear and mononuclear cells.7 However, chronic exposure of these cells to morphine or endorphin for 48 hours leads to a 60% suppression of superoxide synthesis by peripheral blood mononuclear cells, and this effect can be blocked by naloxone.5 It now appears that most of these effects are mediated via the µ-opioid receptor. Thus, recent studies have shown that chronic morphine administration to mice results in a twofold to threefold inhibition of thymic, splenic, and lymph node cellularity, inhibition of thymic-lymphocyte proliferation, inhibition of interleukin-2 and IFN-γ synthesis, and activation of macrophage tumor necrosis factor-α and nitric oxide synthesis. These effects are abolished in µ-opioid receptor knockout mice.6 Overall, these data suggest that chronic opiate abuse might lead to impaired host defense against viral infection or replication, as seems to be the case with HIV infection.9–11

In addition to the effects of opiates on viral replication, opiates may also enhance liver injury and the development of hepatic fibrosis. Injection of morphine causes an acute increase in liver enzymes and a decrease in hepatic glutathione (GSH) synthesis.12 Recent studies have shown that the opioid antagonist naltrexone decreases liver injury in rats and mice with acute biliary obstruction.13,14 Jaume and colleagues15 have also shown that opioid receptor blockade reduces Fas-induced hepatitis in mice and that treatment with morphine enhances mortality. These studies suggest that these effects may be secondary to an altered redox state in the liver cell but also suggest that morphine increases the secondary liver injury that follows viral infection.

Although opiates may increase viral replication and injury, do they have any effect on the development of hepatic fibrosis? While this is still under investigation, data are beginning to suggest that naltrexone has a marked anti-fibrogenic effect in biliary cirrhosis and that this may be secondary to changes in the redox state of the hepatocyte.16 Thus, the chronic administration of naltrexone almost completely prevents the development of hepatic fibrosis in a rat model of biliary cirrhosis (unpublished). Whether the converse is true (ie, does chronic morphine abuse accelerate hepatic fibrosis?) is still awaiting investigation.

Concluding Remarks

Thus, based on current and previous studies with respect to the liver, it appears that chronic opiate abuse leads to: 1) increased risk of viral infection; 2) enhanced viral replication; 3) increased liver injury; 4) decreased hepatic glutathione levels; and 5) increased hepatic fibrosis. The growing implication from these and other studies is that continued opiate abuse leads to enhanced viral replication, liver injury, and hepatic fibrosis. Further studies are required to determine whether these effects occur in humans as well.

Therapy for HCV has been successful even when patients have not abstained from continued drug or alcohol use or are receiving daily methadone. Methadone treatment has been shown to reduce risk behavior that can spread HCV or impair adherence to treatment, and it is not a contraindication to HCV treatment. However, cur-
rent data suggest that elevated levels of HCV replication, caused by opiates and their withdrawal, may impair responses to interferon. In light of the effect of opiates on HCV replication and immune responses, it is clear that studies on viral kinetics during methadone therapy are warranted to ensure comparable responses to current regimens in patients with ongoing opiate use.

References