

1. An association between *Helicobacter hepaticus* infection and hepatocellular tumors in susceptible strains of laboratory mice has been proven. What evidence would be needed to establish a link between enterohepatic *Helicobacter* species (i.e. non-gastric *Helicobacter* species) infection and liver cancer in humans? Describe the kinds of studies and findings that you think would lead the International Agency for Research on Cancer to include infection with enterohepatic *Helicobacter* species along with infection with *Helicobacter pylori*, schistosomes, and liver flukes as being carcinogenic to humans (Group 1).

2. In an editorial accompanying the paper by Kirk Mauer et al. titled "Identification of cholelithogenic enterohepatic *Helicobacter* species and their role in murine cholesterol gallstone formation" (Gastroenterology 2005), Alan Hofmann wrote:

Mauer et al. show convincingly that infection with a single strain of *Helicobacter* (*H. bilis*) or 2 strains (*H. hepaticus* and *H. rodentium*) of enterohepatic *Helicobacter* accelerates the formation of cholesterol gallstones in mice fed a lithogenic diet. The article sheds little light on the mechanism of this effect. No data are provided on biliary lipid composition or on the activities of the transporters encoded by the *Lith* genes. Pathologic changes in the intestine, liver, and biliary tract are described sketchily. Although *Helicobacter* DNA was not present in bile at the time of sampling (16 weeks), the gallbladder mucosa was inflamed, with "hyalinosis, intestinal metaplasia, eosinophilic inflammation, hyperplasia, and dysplastic change" being observed. Presumably, future articles from these laboratories will provide detailed information on these key features of the disease model.

Describe one experiment that you think would help establish the mechanism by which enterohepatic *Helicobacter* infection contributes to cholesterol gallstone disease in mice fed a lithogenic diet. Be sure to state the hypothesis your experiment will test.

3. It has been suggested that hepatocellular tumors in rodents treated with chemical carcinogens develop from foci of altered hepatocytes. These foci have altered phenotypes based on their histochemical appearance (i.e. increased γ -glutamyl transferase [γ -GT] expression). However, the functional changes in these foci that confer malignant potential are not understood. What do you think are the main advantages and limitations of using (A) genomic and (B) proteomic analysis to characterize these altered foci? What are your main technical and conceptual concerns (i.e. potential problems) about using these approaches to ascertain the role of altered foci in hepatic carcinogenesis?

4. What do you think is the best currently available animal model for human viral hepatitis leading to hepatocellular carcinoma? You should define what you mean by best, and can limit your comparison to hepatitis B virus, hepatitis C virus, or both. Be sure to explain your reasoning.

5. It has been argued that the only real value of randomized, controlled chemoprevention studies is that they can demonstrate sufficient weight of evidence to justify committing immense resources to primary prevention efforts. Do you think that intervention with micronutrients or pharmaceutical agents to prevent hepatocellular carcinoma in parts of the world with high rates of aflatoxin exposure and hepatitis virus infection is worthwhile, or do you think that prevention should focus exclusively on eliminating food contaminants and vaccination? Explain your reasoning.