Molecular and Biological Approaches to Understanding Hepatitis C Virus

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The development of potent vaccines against hepatitis A virus and hepatitis B virus has made the prevention of hepatitis A and hepatitis B a possibility. However, hepatitis C virus remains a world-wide threat and its prevention by vaccination a desirable but difficult goal. Our inability to replicate the virus in cell culture in a reproducible and useful manner and the limited host range of HCV, which includes only humans and chimpanzees, make the study of this virus difficult. Development of vaccines against HCV is further complicated by its genetic heterogeneity. We have approached the problem by combining molecular studies of the viral genome with biological studies of the virus in chimpanzees. We have constructed infectious cDNA clones of HCV that can be tested for viability by ultrasound-guided percutaneous intrahapatic transfection of chimpanzees. Using this technique, we have confirmed the observation of Charles Rice and his colleagues that the near consensus sequence of HCV is necessary for viability and that most, if not all, of the 3' non-coding region of HCV cDNA is required for infectivity in vivo. Although initial studies were with the genotype 1a H strain of HCV, we have extended our studies to other genotypes. This has been facilitated by the preparation of reference pools of genotypes 1b, 2a, 2b, 3a, 4a and 5a in chimpanzees. Plasma pools from such infected chimpanzees have been titered for infectivity and have served as a source of virus for additional cloning and molecular studies. For example, a viable chimeric virus has been constructed from cDNA comprising the open reading frame of genotype 1b and part of the the 5' and 3' non-coding regions of genotype 1a. Other chimeric viruses, consisting of sequences from HCV genotype 2a and from genotype 1a have been constructed and are under study. Monoclonal HCV, derived from chimpanzees transfected with infectious HCV cDNA, has been useful for studying the emergence of variant viruses to form quasispecies in vivo. Pools of such monoclonal virus can be used as challenge virus in vaccination studies of chimpanzees to dissect the nature of protection and the role of heterogeneity in the escape of HCV from immunological control.