logical studies (5) have eliminated chronic acalculous cholecystitis as a pathological entity characterized by chronic inflammation and fibrous and muscular thickness of the gallbladder wall.

Undoubtedly, chronic right upper quadrant pain in the absence of gallstone disease is multifactorial. Sludge and gallstones must be eliminated by ultrasonography; abnormal bile should be sought by examining the duodenal aspirate for the presence of cholesterol crystals. Overt causes of impairing gallbladder motor function (e.g., progesterone, obesity and diabetes) must be eliminated (10). Only then will impaired gallbladder evacuation be properly related to symptoms and outcome.

In the interim the ejection fraction ascertained by quantitative cholescintigraphy is a reasonable tool for identifying disordered gallbladder motility and relating this to biliary symptoms. Even pain relief soon after surgery is an imperfect endpoint because of placebo effect. The advent of laparoscopic cholecystectomy will be a temptingly easy solution to difficult cases. Before becoming too haphazard, a more complete, careful evaluation of gallbladder function and contents is essential. The once elusive test(s) to diagnose acalculous biliary pain are being clarified. Cholescintigraphy may yet provide the best bet.

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GENETIC STRUCTURE AND HETEROGENEITY OF HEPATITIS C VIRUS: A VACCINE IMPEDIMENT?

Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, et al. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 1991;88:2451-55.

ABSTRACT

The nucleotide sequence of the RNA genome of the human hepatitis C virus (HCV) has been determined from overlapping cDNA clones. The sequence (9379 nucleotides) has a single large open reading frame that could encode a viral polyprotein precursor of 3011 amino acids. While there is little overall amino acid and nucleotide sequence homology with other viruses, the 5′ HCV nucleotide sequence upstream of this large open reading frame has substantial similarity to the 5′ termini of pestiviral genomes. The polyprotein also has significant sequence similarity to helicases encoded by animal pestiviruses, plant potyviruses, and human flaviviruses, and it contains sequence motifs widely conserved among viral replicases and trypsin-like proteases. A basic, presumed nucleocapsid domain is located at the N terminus upstream of a region containing numerous potential N-linked glycosylation sites. These HCV domains are located in the same relative position as observed in the pestiviruses and flaviviruses and the hydrophobic profiles of all three viral polyproteins are similar. These combined data indicate that HCV is an unusual virus that is most related to the pestiviruses. Significant genome diversity is apparent within the putative 5′ structural gene region of different HCV isolates, suggesting the presence of closely related but distinct viral genotypes.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Shimotohno K. Sequence diversity of hepatitis C viral genomes. Mol Biol Med 1990;7:495-501.

ABSTRACT

The nucleotide sequences of cDNAs (275 base-pairs) in the non-structural protein 5 regions of Japanese isolates of hepatitis C virus (HCV-J) from the plasma of 11 patients with non-A, non-B hepatitis and the livers of five patients with hepatocellular carcinoma were analyzed. Approximately 14 to 17% of nucleotide sequences of the HCV-Js examined differed from that of the original isolate in the United States (HCV-US). Furthermore, 2.5 to 11% sequence diversity was found among the HCV-Js. The nucleotide sequences of the HCV-Js showed characteristic common differences from that of HCV-US, although they also showed some random substitutions. Plural HCV-J genomes were found in two of the cDNAs derived from liver specimens, and a deletion of 102 nucleotides was found in the cDNA derived from one plasma specimen. These results suggest that HCV-J is a strain different from the HCV-US and that mutation of the viral genome occurs at as high a frequency as in that of the human immunodeficiency virus.

COMMENTS

Recently, several groups reported the initial structural details of the hepatitis C viral genome (1, 2). Two

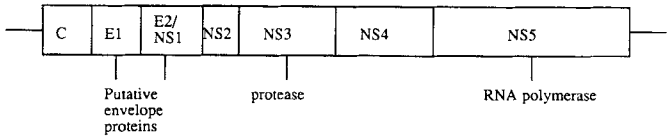

FIG. 1. Hepatitis C virus genomic map. The box represents the open reading frame that would encode the 3011 amino acid precursor protein. Proteolytic cleavages would generate the following putative viral proteins: C = RNA-binding nucleocapsid protein; E1 = envelope; E2/NS1 = envelope/nonstructural protein 1; NS2-5 = nonstructural proteins 2-5.

such reports are reviewed here along with the current knowledge of hepatitis C virus (HCV) genetic heterogeneity. The first paper is from the Chiron group that isolated and cloned the HCV in 1988 and reports the genomic sequence of the virus. Choo et al. isolated and sequenced overlapping complementary DNA (cDNA) clones from a cDNA library prepared from infectious chimpanzee plasma. The sequence determined was 9,379 nucleotides long and contained a single large open reading frame. This open reading frame could encode a polyprotein precursor of 3,011 amino acids that presumably is proteolytically cleaved to form the various viral proteins. Computer analysis of nucleotide and deduced amino acid sequences showed very little sequence homology to other known viruses. Several small portions of the genome had sequence similarity to both human flaviviruses (e.g., yellow fever virus) and animal pestiviruses (e.g., bovine viral diarrhea virus). These clues suggested that the overall genomic structure of these viruses might be similar, and indeed hydropathy plots of the viral polyproteins showed remarkable similarity. The presumed genomic structure of hepatitis C is shown in Figure 1. Structural proteins are located at the 5′ end of the genome and appear to encode nucleocapsid and envelope proteins. Five nonstructural proteins make up the remainder of the polyprotein and are currently named NS1 through NS5. NS3 contains some structural homology to serine proteases such as trypsin and other viral proteases and presumably encodes an enzymatic activity that helps to cleave the large viral proprotein (cellular proteases may also be involved). NS4 contains the antigenic sequence that is currently detected by commercial HCV ELISA assays (C100). NS5 appears to be the viral RNA-dependent RNA polymerase. In addition, the 5′ upstream region preceding the open reading frame has significant homology to animal pestiviruses consistent with this region encoding important translational and/or transcriptional regulatory elements. The overall sequence similarities suggest that HCV is most closely related to pestiviruses.

One of the main questions for vaccine development in human viral disease is the degree of structural heterogeneity between different viral strains. If such heterogeneity is stable it may be possible to prepare polyvalent vaccines such as the trivalent polio vaccine. A more ominous heterogeneity is that which is unstable and even continually evolving, as may be the case for the human immunodeficiency virus (HIV). To begin to understand HCV diversity the second paper used polymerase chain reaction amplification and sequencing of a 275-bp fragment of NS5 (encoding 91 amino acids). Sixteen Japanese patients were analyzed and compared with the original United States isolate sequenced above. The Japanese isolates differed from the original United States isolate by 14% to 17% in nucleotide sequence. Of greater interest, 7 of 91 amino acids in this putative polymerase (NS5) segment were always different as compared with the United States isolate and were essentially identical among the Japanese patients. Scattered single amino acid changes were found in individual Japanese isolates, but overall their homology was much higher in comparison with the United States isolate. Although this paper only looked at a very small portion of the HCV genome it suggests that different viral genotypes exist and raises questions about the effect of genetic heterogeneity on vaccine development.

A thorough review of HCV genetic heterogeneity was recently published in this journal (3) and further develops many of the findings in these papers by comparing all published HCV sequences. Analysis of this sequence data reveals three groups of HCV genotypes (called HCV I, II and III). Multiple genotypes from different HCV groups have been found in Japan and Europe, whereas only group I HCV viruses have been found in the United States. Further sequence data must be obtained from HCV isolates to answer several important questions, which include the following: Are there other groups of HCV genotypes? How rapidly is this virus evolving? Does HCV continue to evolve/mutate in individual patients? Can individuals be infected with more than one genotype? The availability of polymerase chain reaction has made it possible to analyze these issues with a speed and efficiency previously (circa 1985) unimaginable. Ogata et al. (4) analyzed HCV isolates obtained from blood collected in 1977 and 1990 from the same patient (4). The two isolates differed at 123 of 4923 nucleotides (2.5%) in a patient with no known HCV exposures during this 13-yr interval. Assuming that the virus continuously replicated during this time, the authors calculated a mutation rate of approximately 10^{-3} nucleotide substitutions per site per year. This mutation rate is similar to that of other RNA viruses and about a millionfold higher than seen with chromosomal DNA. Viral RNA polymerases lack a proofreading function present in DNA polymerases that may explain these markedly different mutation rates. The clinical implications of such high mutation frequencies are not yet known.

Mutation of particular viral proteins may have far-reaching consequences. The viral envelope protein protrudes from the virion particle and not only mediates important functions such as viral attachment and/or cell entry but also provides the antigenic signature that identifies specific virions to the immune system. Hypervariable regions within the envelope protein have been well defined in the HIV (5-7). These regions may permit escape of new viral strains from immune surveillance.

An evolving body of information on HCV envelope sequences appears to show similar hypervariable regions. The biological implications of such "new envelope" viruses were investigated recently (8). Four chimpanzees were serially inoculated with different infectious strains of HCV. After the first inoculation, each animal had acute hepatitis and anti-HCV develop. After recovery and infection with a different HCV strain, viremia reappeared with infection by the second viral strain (confirmed by polymerase chain reaction and sequencing). Animals simultaneously had elevations in ALT develop, and one had acute hepatitis develop. Two animals were challenged four times with the same results. The authors concluded that infection of chimpanzees with HCV did not provide immunity to heterologous or homologous HCV. Could this be the experimental correlate of patients who have multiple episodes of NANB hepatitis develop?

If this family of HCV is continuing to evolve at a rapid rate the production of effective vaccines could be compromised. The ability to produce an effective HBV vaccine was facilitated by the biology of HBV. HBV uses a different polymerase for replication that may well have a lower mutation frequency. In addition, the HBV genome has severe size constraints with overlapping genes that may not allow replication of viral mutants. HCV may present a vaccine challenge more similar to HIVs than its hepatotropic relatives.

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