CLINICAL MICROBIOLOGY REVIEWS, Oct. 1994, p. 505–532
0893-8512/94/$04.00+0

Vol. 7, No. 4

Hepatitis C: Progress and Problems

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HISTORY

In 1974, Prince and coworkers at the New York Blood Center reported that of 204 cardiovascular surgery patients followed prospectively, 51 patients (25%) developed posttransfusion hepatitis (294). In 36 patients (18% of all patients and 71% of those with posttransfusion hepatitis), this was not caused by the hepatitis B virus (HBV) (294). They concluded "that a large proportion of long-incubation post-transfusion hepatitis is unrelated to hepatitis B, and that control of post-transfusion hepatitis will require identification of a hepatitis virus(es) type C." Similarly, neither hepatitis A nor hepatitis B accounted for the posttransfusion hepatitis in a group of cardiac surgery patients monitored prospectively at the National Institutes of Health (90). A *Lancet* editorial in 1975 coined the term non-A, non-B hepatitis to describe the entity of hepatitis that was neither A nor B, emphasizing that the diagnosis was one of exclusion (13). In 1989, 15 years after the first suggestion that hepatitis C existed, a molecular biological approach was successful where many other techniques had failed and the hepatitis C virus (HCV) was cloned (59, 180). The virus responsible for most posttransfusion hepatitis, and at least a large proportion of sporadic or community-acquired non-A, non-B hepatitis, has thus been identified (12, 334).

VIROLOGY

HCV was isolated and cloned by using a recombinant immunoscreening approach (59). Human factor VIII concentrate that transmitted non-A, non-B hepatitis was inoculated serially into chimpanzees to obtain an infectious plasma pool of $\geq 10^6$ chimp infectious units per ml. Nucleic acids extracted from a crude viral pellet were used to generate a cDNA library that was screened with sera from a patient with posttransfusion hepatitis. A single positive plaque, 5-1-1, was isolated after $\sim 10^6$ recombinant clones were screened. This positive plaque contained a 155-bp insert that was then used as a hybridization

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probe to isolate a 353-bp insert, clone 81. A series of experiments demonstrated that the cDNA hybridized to RNA from infected chimpanzee liver and serum but not to RNA from uninfected liver or to DNA from infected liver. The complete nucleotide sequence of the RNA genome of the original HCV isolate was then determined from overlapping clones. Thus, without specific knowledge of the type of virus involved, viral nucleic acid was cloned from infectious plasma samples.

Classification

The hepatitis C virion is small (30 to 34 nm) and enveloped and has a single-stranded, positive-sense RNA genome. Icosahedron-shaped particles of 33-nm average diameter recovered from HCV-infected plasma (360) may represent intact virions. A lipid envelope is suggested by the chloroform sensitivity of infectious material (30) and by changes in buoyant density with detergent treatment (155, 255). Physicochemical studies indicate that HCV circulates in two forms, consistent with intact virions and nucleocapsids lacking the host-derived lipid envelope (155). Besides lipids, there are exposed sugar moieties on the surface of hepatitis C virions, similar to those on hepatitis B virions (326). There is a single, large, open reading frame that is predicted to encode a viral polyprotein precursor of ~3,000 amino acids (3,010 in the prototypic U.S. strain HCV-1; 3,011 in the Japanese strain HCV-J1 and other Japanese isolates) (60, 161). The structure and organization of HCV most closely resemble those of flaviviruses and pestiviruses. The polyproteins from each of the viruses contain N-terminal structural domains encoding nucleocapsid and glycosylated envelope proteins, followed by nonstructural domains encoding processing and replicative proteins. Whereas the primary sequence of HCV shares little overall identity with that of individual flaviviruses and pestiviruses, the sequence upstream (5′) of the large open reading frame is similar to that of animal pestiviruses. In addition, sections of the polyprotein have amino acid sequence similarities to animal pestiviruses, plant potyviruses, and human flaviviruses (60, 161). The protein processing of HCV is most similar to that of pestiviruses (225). The nucleotide, amino acid, and organizational similarities to the genera *Pestivirus* and *Flavivirus* of the family *Flaviviridae* have resulted in the proposal that HCV belongs to a separate genus of the family.

Viral Replication

The HCV genome is a positive-sense, single-stranded RNA. Both genomic (plus-strand) and replicative (minus-strand) forms are detected in infected liver tissue by PCR (98, 362) and in situ hybridization (15) and, by some investigators, in serum and peripheral blood mononuclear cells (98, 256, 391). Detection of replicative forms in serum and peripheral blood mononuclear cells may be a PCR artifact (241) since only positive-strand RNA was detected following strand-specific reverse transcription of serum nucleic acids (318). In chimpanzees, HCV RNA was detected in the cytoplasm of most hepatocytes by in situ hybridization 2 days after inoculation with HCV-infected serum (265) and was present in the serum by 3 to 7 days (2, 20, 88, 343, 344). Hepatocyte HCV RNA remained detectable during the period of biochemical hepatitis, evidenced by abnormally elevated hepatic aminotransferase levels (265). There was no apparent correlation between detectable intrahepatic HCV RNA and hepatocyte necrosis, suggesting that the virus may not be directly cytopathic.

TABLE 1. Putative proteins and sequence variability in HCV

Genomic region	Putative protein(s) and/or activities[a]	Sequence variability (minimum % similarity)[a]	
		Nucleotide	Amino acid
5′ Untranslated		90	
Nucleocapsid or core (C)	Nucleocapsid (core) protein	81	90
Envelope (E1)	Envelope glycoprotein	56	49
Envelope (E2-NS1)	Envelope glycoprotein	62	70
Nonstructural (NS2)	Metalloproteinase (?)	57	56
Nonstructural (NS3)	Serine proteinase, nucleoside triphosphatase, helicase (?)	70	80
Nonstructural (NS4)	? (Hydrophobic)	65	50
Nonstructural (NS5)	RNA-dependent RNA polymerase (?)	72	71
3′ Untranslated		26	

[a] Data are from references 31, 41, 80, 131, 354, and 358.

Viral Proteins

Although the virus has not been cultured, expression of viral sequences in a variety of systems has permitted tentative identification of many of the proteins encoded by the single open reading frame, including nucleocapsid (core [C]), envelope, and nonstructural proteins potentially involved in viral replication (Table 1). Currently, it appears that three putative viral structural proteins, the core protein p16-22 (encoded in the C region) and two envelope glycoproteins, gp33-35 (E1 region) and gp70-72 (E2/NS1 region), are processed by cellular signal peptidase cleavage of the polyprotein (Fig. 1). In mammalian expression studies, the core protein, which has RNA binding activity (324), was initially cytoplasmic (335, 341) and localized to the endoplasmic reticulum (324, 335) but was targeted to the nucleus by 6 days (341), whereas transport to a nuclear localization was observed earlier in insect cells (186). When both E1 and E2 were expressed in insect cells, using a baculovirus expression system, or in mammalian cells with a recombinant vaccinia virus vector system, the E1 and E2 proteins were glycosylated and formed a complex (186, 303). Purified E1-E2 complexes were recognized at high frequency by sera from HCV-infected persons (213, 303). In addition, sera from all (20 of 20) immunocompetent HCV-infected patients reacted against vaccinia virus-expressed native E1 and E2 antigens, whereas only 55% (11 of 20) reacted against yeast-derived E1 and E2 antigens (213), indicating that glyco-

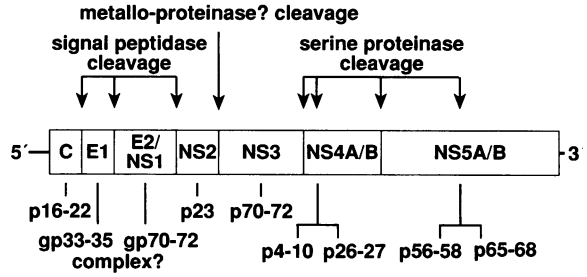

FIG. 1. Putative polyprotein encoded by the HCV genome with potential cleavage sites and resultant proteins. Data are from references 80, 114, 115, 131, 186, 303, and 335.

sylation and/or complex formation was important in generating the antigenic epitopes.

Two virus-encoded proteinases are required for additional processing of the polyprotein. A zinc-dependent metalloproteinase activity (131), encoded by the NS2-NS3 region, is essential for cleavage at the NS2-NS3 boundary in *trans* (115). A virus-encoded serine protease activity, located in the N-terminal portion of the nonstructural protein p70 from the NS3 region (80, 114), apparently cleaves the NS3-NS4 boundary in *cis* (370) and the NS4-NS5 boundary, as well as internal sites in the NS4 and NS5 regions (NS4A-NS4B, NS5A-NS5B) in *trans* (80, 114, 370). A C-terminal 33-amino acid sequence of the NS4A protein is required for some cleavages (85), and this peptide sequence can activate the NS3 protease in *trans* (85). By analogy with other members of the *Flaviviridae*, the nonstructural proteins are considered likely to be involved in viral replication. The NS3-encoded protein p70 has polynucleotide-stimulated nucleoside triphosphatase activity (358) and putative RNA helicase activity in addition to serine protease activity. The NS5 region bears sequence similarity to other RNA polymerases (138); however, the exact functions of NS4 and NS5 proteins in the viral replication complex have not been elucidated.

SEQUENCE DIVERSITY

The ability to sequence specific areas of the HCV genome has allowed comparison of isolates from different individuals (Table 1) (60, 161). On the basis of sequence analysis, there exist at least six major genotypes (genotypes 1 to 6), with two or more related subtypes (e.g., subtypes 1a, 1b, and 1c) within the major genotypes (354). Although there is no standard genotype nomenclature at present, published correspondence suggests that the classification of Simmonds et al. (352) has been accepted by many investigators (351). The precise implications of finding a particular genotype in any individual person are not yet elucidated, although ongoing mutation may be important in immune escape mechanisms (see next section). Furthermore, viral genotype and viral titer affect responsiveness to therapy with interferon (see below). Thus far, however, neither genotype nor viral titer has been unequivocally linked to disease progression (411). Individuals can be coinfected with different genotypes (156), particularly if they have experienced multiple exposures to HCV infection (353). In one patient, genotyping permitted diagnosis of superinfection with one genotype in a patient chronically infected with a different genotype (156).

There is an overall sequence similarity of ~70 to 80% between the major genotypes of HCV (31; see also Table 1). The 5′-untranslated region contains the highest degree of sequence conservation, with ≥90% similarity overall (40, 193); however, the region consists of highly conserved domains interspersed with variable domains (40). In other viruses, similar conserved 5′-untranslated regions have regulatory importance in replication of the viral genome or expression of viral genes. Elements in the 5′-untranslated region of genomic length HCV RNA that repress translation have been described (413). Such elements may not be present in subgenomic length HCV RNA species (125), thereby potentially allowing efficient translation and expression of viral genes.

Other regions of the HCV genome demonstrate greater degrees of diversity than the 5′-untranslated region (41, 351, 352, 354). The core region is relatively well conserved, with nucleotide sequence similarity ranging from 81% (maximum diversity) to 88% (minimum diversity) between isolates of different genotypes (354). The E1 and NS5 regions are less well conserved. Thus, isolates from different genotypes exhibit 53 to 69% sequence similarity in the E1 region and 56 to 72% in the NS5 region (354). Within a specific genotype, subtype nucleotide sequence similarity is higher, ranging from 88 to 93% (core), 68 to 79% (E1), and 75 to 86% (NS5 region) (354). Within a subtype, sequence similarity among isolates ranges between 88 and 99% in the core, E1, and NS5 regions (354). Sequence data from other major areas of the genome are less extensive, except for the amino-terminal end of E2/NS1, which constitutes a hypervariable region. A segment of 28 amino acids in this region demonstrates more than 50% variation in different isolates. This hypervariable region, which includes <1% of the total amino acids in the polyprotein, contained 8 of the 44 amino acid substitutions observed in an experimentally infected chimpanzee over 8 years (277). Even more striking, 9 of 13 amino acids in the E2/NS1 hypervariable region were changed in strain HCV-H when compared over a 13-year period in patient H (272). The average mutation rate for the entire HCV genome has been calculated by comparing the sequence of the chimpanzee and HCV-H isolates obtained at different times. Rates of $\approx 1.4 \times 10^{-3}$ and $\approx 1.9 \times 10^{-3}$ base substitutions per nucleotide per year have been predicted (272, 277). The mutation rate of most RNA viruses ranges from 10^{-1} to 10^{-4} substitutions per site per year, considered to be a reflection of the lack of proofreading ability of the RNA-dependent RNA polymerases. Like other RNA viruses, HCV genomes in a single patient can be a mixed population (231, 257), with a dominant sequence accounting for the majority of isolates. Other variants, present in minor proportions, then have the potential to become dominant if selection pressures are applied.

IMMUNOLOGIC RESPONSE TO HCV INFECTION

The extremely high rate of amino acid change in the hypervariable region of the E2/NS1 segment suggests that functional and structural constraints on the encoded protein are low, as predicted by analysis of the region (363, 397). In the analogous segment of flaviviruses (NS1) and pestiviruses (E2), antigenically distinct variants are favored by selective immune pressures. A similar immune selection of HCV variants is observed (159, 342, 363, 397). Clinically, changes in the hypervariable region may be silent (178, 261) or associated with distinct episodes of hepatitis (181, 319, 397). Antibodies that prevent initiation of replication in vitro, and therefore have the potential to function as neutralizing antibodies in vivo, have been detected (342). Sequence divergence over time resulted in the emergence of neutralization-resistant variants (342). Immune selection of variants to escape from neutralizing antibodies is also suggested by the observation that the HCV hypervariable region in a patient with agammaglobulinemia showed no mutations over a 2.5-year period (179). Together, these findings support the hypothesis that HCV sequence variation contributes to the failure of the immune response to clear the infection and develop protective immunity.

The immunologic reaction to HCV infection also includes cell-mediated responses. HCV-specific cytotoxic T lymphocytes have been detected in the livers of chimpanzees with acute and chronic infection (82) and in chronically infected patients (174, 175, 348). Both core (nucleocapsid) and envelope proteins are recognized (174, 348). Cytotoxic T cells that recognize the nucleocapsid protein can also be generated in vitro (163). Whether host-derived cytotoxic responses are important in limiting HCV infection is unresolved. Other identified immunologic responses include in vitro proliferation

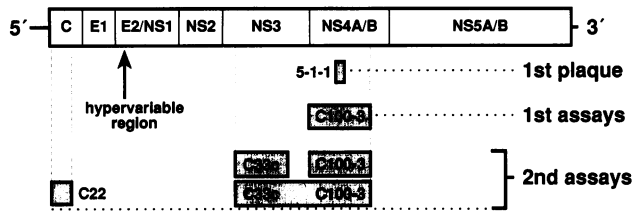

FIG. 2. Sequences expressed in first- and second-generation assays for antibodies to HCV and relationship of hypervariable region to putative polyprotein.

of peripheral blood mononuclear cells from HCV-infected patients cultured with viral antigens (93, 331) and immunogenic B-lymphocyte epitopes on the nucleocapsid and envelope proteins (5, 250, 304). Thus far, the exact role(s) of immunologic responses to the virus by the host in the generation of liver disease and its persistence has not been defined. However, the observation that treatment with corticosteroids decreased hepatic aminotransferase levels, even though viral titers increased in serum (99, 222), suggests a role for immune-mediated hepatocyte damage. In addition, exacerbation of chronic liver disease occurs with cessation of immunosuppression in individual patients (86, 117). Furthermore, in HCV-positive, human immunodeficiency virus (HIV)-positive persons (233) and in those with gamma globulin deficiencies (24, 198), liver disease can be aggressive. Although the exact mechanism of liver cell damage in HCV infection is not known, these observations indicate that immunoglobulin and $CD4^+$ T lymphocytes are not essential for progression.

Whereas specific immunologic reactivity to HCV infection has been documented, the immune response is apparently insufficient to overcome the infection in many individuals and does not result in protective immunity. Thus, chimpanzees may develop recurrent hepatitic responses and viremia when sequentially inoculated with different strains of HCV (87). Reinfection of the chimpanzees, not reactivation of previously occult virus, was demonstrated by PCR sequencing of the virus strain after inoculation (87). A similar sequence of events may explain multiple episodes of hepatitis in polytransfused individuals (183). If the responses of humans are similar to those of chimpanzees, then clearance of HCV from the blood and liver is not associated with protective immunity.

DETECTION OF INFECTION

Prevention of posttransfusion hepatitis C by identifying blood donor specimens capable of transmitting the virus to recipients was a major goal once the virus was isolated. Antibodies that recognize HCV protein epitopes have been detected in blood donor specimens and used to identify patients with HCV infection. The original fusion protein, expressed in yeasts, consisted of 363 virus-encoded amino acids from the NS4 region and was used in the first-generation enzyme-linked immunosorbent assay (EIA-1) for the detection of antibodies to HCV epitopes (Fig. 2). This assay was able to identify HCV in the majority of transfusion recipients developing posttransfusion non-A, non-B hepatitis (9, 382). In addition, donors implicated in the transmission of non-A, non-B hepatitis were also identified (9, 382). Clearly, some donors and recipients were negative for HCV infection by the first-generation assay (9, 382). Furthermore, although many patients with chronic hepatitis C had antibodies that reacted with the C100-3 amino acid sequence, a proportion remained negative. The sensitivity of first-generation assays was dependent on the population being studied, being greater (80 to 90%) in persons with parenteral risk factors and elevated hepatic aminotransferase levels suggesting chronic hepatitis (32) and lower (~60%) in volunteer blood donors (395). False-negative results with the first-generation assay were, in part, explained by sequence variation (243, 259). Antibody positivity was also delayed when only the C100-3 sequence was used for antigen expression, with seroconversion occurring 2 to 26 weeks after the onset of hepatitis (380), decreasing the usefulness of the first-generation assay in the diagnosis of acute hepatitis C. The first-generation assay, therefore, lacked sensitivity, particularly for early recognition of acute disease.

The initial assays were also lacking in specificity. Thus, in one study, 44 of 14,068 blood donors were positive by EIA-1; however, 29 of 44 serum samples were negative by confirmatory recombinant immunoblot assay (168). Sera with antibodies that cross-reacted with superoxide dismutase, the fusion partner used for expression of viral sequences in yeasts, accounted for some of the lack of specificity and were identified in a recombinant immunoblot assay (first generation). False-positive results with antibody assays for HCV infection have also been associated with stored and/or "aged" sera (212), sera from tropical communities (369), and following influenza vaccination (219). Hyperglobulinemia and rheumatoid factor (anti-immunoglobulin) also resulted in false-positive anti-HCV tests with first-generation assays (239, 332). Attempts to improve both the specificity of blood donor screening and the sensitivity of detecting patients with HCV infection have resulted in a number of newer assays.

Second- and Third-Generation Assays

Other putative HCV proteins were examined for potential capacity to detect acute infection earlier and chronic infection with greater reliability. Linear peptides encoded in the putative nucleocapsid or core (C) region and the E2/NS1 region are recognized by human antibodies (57, 264, 321, 397). Within each region, some sequences are recognized more commonly, suggesting that they are immunodominant (264, 321, 397). In addition, serologically distinct responses to the putative core protein were demonstrated in patients with genotypically different viral isolates (218). These antibodies recognized peptides encoded in a variable segment of the otherwise highly conserved region. In comparison with first-generation assays, synthetic peptides from the core region allowed earlier detection of HCV infection (a mean of 6 weeks after the onset versus a mean of 9 weeks) (380). HCV RNA is also more likely to be detected in samples containing antibodies to core sequences (62).

Sequences from the NS3 region (C33c) appear to be immunogenic in the majority of those with HCV infection (56). Furthermore, the C200 polypeptide that includes C33c, C100-3, and the intervening region (NS3 and NS4 sequences) was the most effective at blocking labeled antibody binding to HCV antigen(s) in hepatocytes (176). These peptide sequences have been included in the second-generation EIA (EIA-2) and immunoblot assays to provide multiple separate antigenic epitopes (Fig. 2) (56). Inclusion of native envelope protein antigens may increase sensitivity and permit earlier detection of seroconversion in future assays (213). Thus, sera react against E1 and E2 antigens expressed in yeasts less commonly than when expressed in mammalian cell systems, in which glycosylation and complex formation can occur (213).

Second-generation assays have increased sensitivity (99%) in identifying blood donors with hepatitis C viremia (395) and

in diagnosing both acute and chronic hepatitis C infection (32, 195, 295). Furthermore, HCV antibodies were detected with 100% sensitivity in 184 hemophiliacs and 31 other patients with chronic hepatitis and HCV viremia (32). In addition, improved specificity has also been demonstrated by some investigators. Fewer EIA-positive blood donors were negative on confirmatory assay: 22 of 70 (31%) by EIA-2 compared with 29 of 44 (66%) on EIA-1 screening of 14,068 donors (168). Overall, the specificity of current assays is >99.5% (195, 327). Cross-reactivity to the superoxide dismutase fusion partner of C100-3 expressed in yeasts and to proteins in lysates of yeasts and *Escherichia coli* may be minimized by including these proteins in the diluting solutions, thereby preventing attachment of the nonspecific antibodies to the immobilized, specific proteins (295). False-positive rates may remain high with sera collected in Africa, even when analyzed by second-generation assays (44). New third-generation assays contain an additional antigen from the NS5 region. Improvement in sensitivity and/or specificity was not demonstrated in a study of French blood donors with evidence of hepatitis (elevated levels of the hepatic enzyme alanine aminotransferase [ALT] in serum) that compared second- and third-generation assays (228).

The prevalence of anti-HCV reactivity (EIA-2) in the U.S. blood donor population is 0.37% (153 of 41,250) (327) at the Puget Sound Blood Center and varies from 0.33 to 0.70% (15 of 4,517 to 30 of 4,089) in volunteer donor centers in Missouri and California (168). Since prospective donors are currently screened for "high-risk behavior" and known history of hepatitis and repeat donors have been previously tested for anti-HCV positivity, this likely represents the population with the lowest prevalence of HCV infection. Rates of positivity are similarly low in blood donors in the United Kingdom (0.33%, 340 of 310,203) (78) but higher in blood donors in Japan (1.3%, 212 of 16,500) (395) and in the general population in Taiwan (2.5%, 37 of 1,500) (339). Other populations in the United States may have considerably higher rates of positivity than volunteer blood donors. For example, of 626 consecutive patients at a Dallas, Tex., orthopedic clinic, 43 (6.9%) were anti-HCV positive (123), whereas the prevalence among Dallas blood donors is similar to that elsewhere in the United States (37 of 5,232 in September to December 1993 [0.7%]). The true prevalence of HCV infection may therefore be substantially higher than the 0.3 to 0.7% observed in blood donors.

Anti-HCV antibodies persist in the serum in most patients (12, 76) but may become undetectable spontaneously or following interferon therapy (12, 76, 325, 372, 417). Thus, in a follow-up study of 81 patients who developed non-A, non-B hepatitis after receiving HCV-infected immune globulin, 52 of 56 (93%) were anti-HCV positive 6 to 12 months after infection and 45 of 65 (69%) were positive 9 to 10 years after infection (76). Loss of antibody is more common in those who have resolving disease clinically (12, 76, 372), but it is also observed in conjunction with development of immunocompromise from HIV infection (48, 213, 244, 302). Loss of reactivity to only C100-3, the single antigen expressed in the initial assays, is more common than loss of reactivity to all antigens (325, 372).

HCV RNA and Detection of Infection

HCV sequences can be detected either by standard PCR amplification techniques, using conserved oligonucleotide primers followed by hybridization of a labeled probe to internal sequences, or by "nested" or two-stage PCR amplification with external and internal primers and visualization after gel electrophoresis. As with all PCR techniques, detection of HCV RNA requires strict adherence to procedures designed to reduce contamination. Special problems have been encountered in PCR amplification of HCV RNA because the sequence is variable. Oligonucleotide primers from highly conserved regions such as the 5′ noncoding sequence are more reliable (39, 47). Correct collection and storage of samples used for PCR amplification are also important (65, 68, 394). Heparinized plasma is not suitable and serum samples must be centrifuged within 2 h of clot formation to maintain HCV RNA integrity (68, 394). False-positive and false-negative results may be common and render interpretation difficult. Thus, when tested with coded samples, only 5 of 31 (16%) laboratories performed without error (420). By using PCR amplification, HCV RNA has been detected in liver tissue (33, 98, 137, 398) and in peripheral blood mononuclear cells (28, 139, 391, 424) as well as in serum. Faster and simpler methods of detecting and quantifying HCV RNA are currently being explored (107, 141, 208, 383). Methods that depend on signal amplification rather than PCR amplification (68, 222) may be more reliable although less sensitive.

TABLE 2. Viremia in anti-HCV-reactive blood donors[a]

RIBA reactivity[b]	No. (%) of HCV RNA-positive blood donors/no. tested		
	Normal ALT level	Abnormal ALT level	ALT level not stated
Multiantigen (with C22 and/or C33c)	35/70 (50)	130/137 (95)	209/267 (78)
Single antigen (C22 or C33c)	8/71 (11)	11/29 (38)	16/507 (3)
Single antigen (C100-3 and/or 5-1-1)	0/17	0/11	0/202
Negative	0/64	0/15	

[a] Data are from references 35, 78, 240, 313, 327, 340, and 419.
[b] RIBA, recombinant immunoblot assay.

A major focus of measurement of HCV RNA has been correlation with antibody screening tests and hence identification of potentially infectious blood donations. It rapidly became clear that whereas first-generation assays were able to identify blood donations with antibodies to HCV, the positive specimens were not all associated with transmission of hepatitis (106, 381). Confirmatory testing, with a second-generation recombinant immunoblot assay, was more specific (381). In contrast to the lack of specificity of early screening assays, the presence of HCV RNA is associated with transmission of HCV (106, 381, 387, 398). In a prospective study of posttransfusion hepatitis carried out before screening tests were licensed, anti-HCV antibodies and HCV RNA were measured in serum samples obtained from 1,100 donors and 300 recipients (106). Of six donor samples (0.6%) repeatedly reactive in a first-generation assay, only one (17%) transmitted non-A, non-B hepatitis to a recipient. HCV RNA was detected by PCR in the serum of the transmitting donor but not in the sera of the other five donors, demonstrating that the presence of detectable viral sequences was a better predictor of infectivity.

The finding of EIA-2 reactivity in blood donors does not correlate absolutely with hepatitis C viremia either (8, 69, 104, 357, 403). Supplementary testing with recombinant immunoblot assays, however, provides more accurate prediction of infectious samples (Table 2). Thus, HCV RNA is usually detected (mean, 79% positive) if there is reactivity to at least two separate antigens (35, 78). With evidence of hepatitis (abnormally elevated ALT levels), the rate of positivity is

greater (≥95%) (240, 340, 419) than when hepatic enzymes are normal (≤54% positive) (240, 313, 340). Viral titers tend to be higher in patients with chronic liver disease than in asymptomatic blood donors (119), with acquisition of HCV from blood transfusion (188), and when there is immunosuppression by corticosteroid treatment (99, 222), after transplantation (54), or from coinfection with HIV (338). Both increased (160) and decreased (222) viral titers have been observed in relation to increasing severity of liver disease, indicating that there are a number of factors interacting to determine circulating levels of virus. Viremia is less common when reactivity is to a single antigen from the core or NS3 region, particularly if ALT levels are normal (240, 313, 327). HCV RNA was not detected in samples from anti-HCV-positive blood donors when reactivity was limited to C100-3 and/or 5-1-1 antigens (35, 78, 240, 327, 340, 419) or when supplementary testing was negative (240, 313, 327, 340, 419), regardless of ALT levels (Table 2). Together, these data suggest that the newer assays will be an effective screen for detecting blood donations capable of transmitting HCV. However, when 108 donors with ALT concentrations of >100 IU/liter were studied, 19 were PCR positive for HCV RNA but only 13 had antibodies detected by EIA-2 (357), implying that large-scale screening by PCR might be needed to eliminate posttransfusion hepatitis C if newer serologic assays continue to miss some viremic donors.

TRANSMISSION OF INFECTION

Transfusion of infected blood and blood products transmits HCV, and hepatitis C infection accounted for 80 to 90% of posttransfusion hepatitis in the United States (1, 12, 173), Europe (110, 235, 372), and Asia (217, 393) before the introduction of anti-HCV screening. Other "parenteral" risk factors for HCV infection include injection drug use (12, 154, 237), tattoos (122, 154, 169, 237), needle stick accidents (165, 254), participation in ritual ceremonies, and use of folk remedies (16, 166). Transplantation with organs from HCV-infected donors can transmit HCV (285, 286, 315) but does not always do so (390). Thus, none of six recipients of kidneys from four anti-HCV-positive, HCV RNA-positive donors became infected, as evidenced by negative anti-HCV and no HCV RNA (390). Perfusion of kidneys with subsequent removal of contaminating blood may explain the difference in transmission. Corneas, which are avascular, may not transmit hepatitis C either, since no HCV RNA was detected in corneas from HCV RNA-positive individuals (191).

HCV infection has been prospectively identified in potential blood donors since the introduction of routine screening, which was in mid-1990 in the United States. Before the introduction of anti-HCV testing, elevated ALT levels and anti-hepatitis B core antibody positivity were used as surrogate markers for blood donations likely to transmit hepatitis. However, HCV infection and elevated aminotransferase levels are not synonymous in blood donors. ALT levels were monitored for 6 months and were repeatedly normal in 5 of 50 (10%) volunteer blood donors with HCV infection in one study (237). Furthermore, although many blood donors with HCV infection may have elevated ALT levels, the converse is not true (158, 379). Two U.S. studies have examined the incidence of HCV infection in blood donors with elevated ALT levels, suggesting the possibility of HCV infection (158, 379). Anti-HCV antibodies were confirmed in only 17 of 100 and 11 of 101 donors, respectively (158, 379). Furthermore, HCV RNA was detected in only 8 of 11 donors with anti-HCV antibodies and none of 89 seronegative donors (379). Current screening assays, therefore, are both more sensitive and more specific

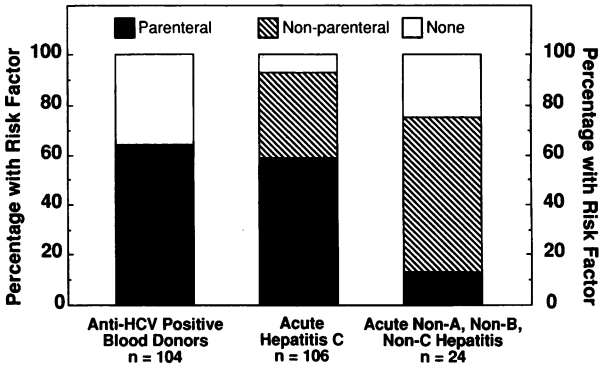

FIG. 3. Parenteral and nonparenteral risk factors in HCV-infected blood donors and patients with acute non-A, non-B hepatitis. Data are from references 12 and 240.

than elevated ALT levels in identifying HCV-infected blood products, and their introduction has resulted in a further decrease in the rate of posttransfusion infection with HCV (19, 77, 268).

Worldwide, injection drug use is probably the most common risk factor for HCV infection, with ~80% seropositivity in this population in the United States (162, 242). In pregnant women in Dallas, Tex., anti-HCV antibodies were positive in 23 of 1,005 (2.3%) when samples were screened by first-generation assays (27). Injection drug use was reported by 10 of 23 (43%) anti-HCV-seropositive women but only 13 of 982 (1.3%) seronegative women. In Australian blood donors, a history of injection drug use was present in 105 of 220 (48%) donors found seropositive for HCV infection but in only 3 of 210 (1%) seronegative controls (154). Similar results were obtained in a study of U.S. blood donors with elevated ALT levels (158). The only risk factor present more often in the anti-HCV-positive group was a history of injection drug use (5 of 17 [29%] anti-HCV positive; 4 of 83 [5%] anti-HCV negative).

In the Sentinel Counties Non-A, Non-B Hepatitis Study, specific questioning of patients with acute hepatitis C elicited the presence of parenteral risk factors in 59% and sexual or household contact with hepatitis in 6% (12). An additional 28% were classified as having a nonparenteral risk factor since they had completed less than 12 years of schooling (Fig. 3), a marker of "low socioeconomic level" and a finding previously shown to correlate with non-A, non-B hepatitis cases when compared with controls (10). In 7%, there were no identifiable risk factors (Fig. 3). For comparison, in 24 patients with acute non-A, non-B hepatitis that was not hepatitis C (non-A, non-B, non-C), no risk factors were identified in 25% and only low socioeconomic level (i.e., nonparenteral risk factor) was identified in an additional 54%. Parenteral risk factors were less frequent in the non-C group (13%), although close contact was similar to those with HCV markers (8%). An investigation of acute non-A, non-B hepatitis in 50 consecutive patients in Dallas, Tex. (150), identified parenteral risk factors in 25 of 34 patients with HCV infection but none in patients without hepatitis C.

Infection in Patients with Repeated Parenteral Exposure

Patients with bleeding disorders or chronic renal failure requiring dialysis are commonly and frequently exposed to blood and blood products. Many hemophiliacs are positive for anti-HCV antibodies. HCV reactivity is associated with both HBV and HIV infections in hemophiliacs (36, 223, 373). In the

United States, anti-HCV antibodies were present in 79% (273 of 345) and 98% (376 of 382) of hemophiliacs without and with HIV infection, respectively (373). A recent prospective study demonstrated that solvent and detergent inactivation of chromatographic factor VIII concentrate was efficacious in preventing anti-HCV seroconversion in 31 patients treated with 41 different lots (230). This approach or a similar viral inactivation process, together with screening donors for anti-HCV reactivity, should prevent transmission of HCV to hemophiliacs in the future.

Unlike factor VIII, intravenous immune globulin generally has not transmitted HCV (368). Consistent with lack of infectivity, HCV RNA was not detected in immune globulin preparations despite the presence of anti-HCV (296, 320). The processing of plasma pools for intravenous immune globulin preparations removes or inactivates HCV (23). Thus, infusion of EIA-1-negative, pooled plasma into chimpanzees resulted in transmission of HCV (23). In contrast, infusion with intravenous immune globulin prepared from the EIA-1-negative, pooled plasma did not transmit HCV. Unfortunately, some preparations of intravenous immune globulin have been implicated in transmission of hepatitis (14, 24, 201, 300, 405), often with progressive disease developing in the recipients (24, 198). Transmission of HCV by immune globulin administered intramuscularly has not been reported. Normally, processing of plasma for intravenous immune globulin administration results in partitioning of most HCV RNA to cryoprecipitate or Cohn fractions I and III (412); apparently, any HCV RNA remaining in the immune globulin preparation is usually inactivated by other processing steps. The apparent outbreak of HCV infection linked to intravenous administration of immune globulin prepared from EIA-2-negative, pooled plasma (14) is of great concern. A number of theoretical explanations for HCV transmission have been proposed (14). These include lack of a viral inactivation step, failure of quality control, and possible inability of normal processing to remove HCV due to high viral titer or lack of anti-HCV antibodies in the plasma pool.

Patients with renal disease on hemodialysis also have the potential to be continually exposed to infectious materials. The prevalence of markers for HCV infection varies widely, depending on geographic origin of the patients and the presence of other parenteral risk factors. In Japan, EIA-2 detected anti-HCV antibodies in 74 of 167 (44%) patients (273). Similarly, in the United States, 31 of 87 (36%) patients were positive by anti-HCV assays (second-generation immunoblot) (127). Reactivity correlates with length of time on dialysis but not blood transfusion history or aminotransferase levels (127, 145). In contrast, only 6 of 79 (8%) Belgian patients were positive for anti-HCV antibodies (EIA-2), with HCV RNA detected in 4 of 79 using nested primers from the 5′ noncoding region (404). Similarly, 28 of 340 (8%) Danish patients on hemodialysis were anti-HCV positive and 27 of 28 had HCV RNA detected (42). Of anti-HCV-negative patients in this study, 7 of 38 (18%) with recently elevated aminotransferase levels and 1 of 16 (6%) with remotely elevated aminotransferase levels were also positive for HCV RNA. None of 36 patients with normal aminotransferase levels and negative for anti-HCV had HCV RNA detected. Altogether, therefore, an additional 8 hemodialysis patients (from 90 anti-HCV-negative patients tested) were infected with HCV, an estimated overall rate of 11% in the Danish hemodialysis population (42). Inclusion of native envelope protein antigens in future assays may permit identification of HCV infection in hemodialysis patients (213) without resorting to measurements of HCV RNA.

The findings that 67 to 96% of anti-HCV-positive dialysis patients have detectable HCV RNA (42, 49, 112, 290, 350, 404) and that serologic positivity correlates with length of time on dialysis suggest that limitation of anti-HCV patients to a particular dialysis station may be useful. However, one study considered the explanation to be nosocomial transmission of HCV by inapparent percutaneous route, since there was infection of dialysis patients at adjacent stations (146). Also suggesting nosocomial spread of HCV, operating room equipment was considered to explain an apparent outbreak of HCV infection in four patients undergoing surgery on a single day (301). Furthermore, in prospective studies of posttransfusion hepatitis rates, hospitalized control patients have developed non-A, non-B hepatitis without transfusions (1), suggesting that there are risk factors associated with transmission of infection in hospitals.

HCV infection is also encountered commonly in recipients of renal transplants (50, 187, 316). In Hong Kong, 23 of 185 (12%) renal allograft recipients were infected with HCV. Anti-HCV antibodies were detected in 19 patients, whereas serum HCV RNA was present without antibody in 4 of the 23 patients (17%) (50). Infection was generally acquired before or at the time of transplantation, again suggesting that measures targeting the hemodialysis population were needed for effective prevention. Similar rates of infection were observed in the United States (187): 25 of 100 renal allograft recipients were infected with HCV before transplantation and 7 of the 25 (28%) were positive for HCV RNA without antibodies. Another six patients acquired HCV infection during the first year after transplantation. Clearly, transplant recipients and other patients will be at increased risk of HCV infection until all routes of transmission of HCV are identified.

Occupational Hazards

Health care workers represent 2% of acute hepatitis C patients (329) and may have higher rates of chronic HCV infection than the general population. Thus, ~2% (8 of 456) of dentists in New York, particularly oral surgeons (9%, 4 of 43), were anti-HCV positive by first-generation immunoblot, significantly more than the control population (0.14%, 1 of 723) (167). The magnitude of the potential risk to health care providers was underscored in a study of the prevalence of seropositivity for HCV in emergency room patients of a large inner-city hospital, Johns Hopkins University Hospital (162). Antibody to HCV (second-generation immunoblot) was present in 18% (458 of 2,523) of patients, with rates as high as 51% in some age-, sex-, and race-defined groups. Similar results have been reported from the Washington, D.C., Veterans Administration Medical Center, with 24% of 450 consecutive inpatients testing positive for anti-HCV by EIA-2 assays (333). Since needle stick exposure may lead to transmission of HCV in up to 10% of instances (infection in 7 of 68 with needle stick from HCV RNA-positive source in Japan) (254), the potential risk of contracting hepatitis C is considerable. However, lower rates of transmission of HCV infection by needle stick accidents to health care workers have been observed in other institutions, 3 of 90 and 0 of 81 employees (128, 356), and antibodies to HCV were found in only 7 of 943 (0.7%) health care workers at the Johns Hopkins University Hospital (367), suggesting that occupational transmission is infrequent. Viral titer differences may explain the disparity.

Other Routes of Transmission

Nonparenteral transmission of HCV may account for infection in patients with sporadic or community-acquired disease who lack classical parenteral risk factors. Alternatively, this

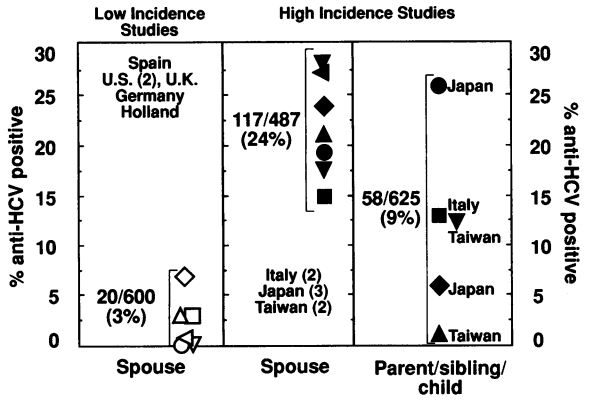

FIG. 4. Incidence of HCV infection in spouse and nonspouse household members. Data for low-incidence studies (left, open symbols) are from references 29, 34, 83, 84, 124, and 210, and data for high-incidence studies (middle and right, solid symbols) are from references 4, 43, 51, 135, 157, 279, and 284. The numbers in parentheses refer to the numbers of studies from each country; each symbol represents a separate study.

may represent inapparent parenteral or percutaneous spread. In patients with either hepatitis B or non-A, non-B hepatitis, multiple heterosexual partners were reported more commonly (hepatitis B patients, 26%; non-A, non-B hepatitis patients, 12%) than in matched controls (7% hepatitis B controls; 1% non-A, non-B hepatitis controls) (10). Anti-HCV antibodies are more common in sexually promiscuous groups, for example, 8% (99 of 1,292) in those attending sexually transmitted disease clinics (400), 6% (20 of 310) in female prostitutes (210), 7% (6 of 88) in clients of prostitutes (210), and 4 to 5% in homosexual men (210, 280), all higher than in volunteer blood donors. The exclusion of subjects with a history of blood transfusion or injection drug use in the determination of prevalence rates in prostitutes, their clients, and homosexual men (210) decreases the likelihood of these risk factors being responsible for the increased seroprevalence.

Heterosexual spouses of anti-HCV-positive hemophiliacs have a low incidence of anti-HCV positivity in some studies (29, 84, 124), with no evidence of transmission in other studies (34, 83) (open symbols, Fig. 4). Coinfection with HIV raises the likelihood of transmission (84), as does the presence of other HCV risk factors (124). Similarly, when the anti-HCV-positive index cases were largely injection drug users rather than hemophiliacs, HCV positivity was lower in stable heterosexual partners (not themselves injection drug users) when the index case was HIV negative (2 of 49, 4%) than when the index case was HIV positive (9 of 98, 9%) (210). Higher rates of HCV infection in household contacts has been observed in studies from Taiwan, Japan, and Italy (solid symbols, Fig. 4), with 15 to 28% of spouses positive for anti-HCV antibody (4, 43, 51, 135, 157, 279, 284). HCV nucleotide sequences from two family members (one child and one spouse) in one study were significantly more homologous to the corresponding patient than to other HCV-infected persons (135) and were identical in three couples in another study (157), suggesting the occurrence of familial transmission in some but not all cases. Other practices, such as folk remedies with percutaneous exposure (166), may explain the increased rate of household and spousal transmission in these areas. Alternatively, higher viral titers may lead to intrafamilial spread as well as to maternal-infant transmission (275).

HCV RNA has generally not been found in body secretions

TABLE 3. Evidence for different modes of transmission of hepatitis C and hepatitis B[a]

Individuals with:	No. (%) positive/no. tested		
	Anti-HCV antibody	HBV marker	HBsAg
Developmental disabilities (outpatients)	0/113	24/113 (21)	3/113 (3)
Down's syndrome (institution)	0/128	116/128 (91)	35/128 (27)
Other handicaps (institution)	0/136	103/136 (76)	8/136 (6)

[a] Data are from references 52 and 203.

(102, 140, 364), although some investigators report its presence (271, 416). When detected, blood contamination may potentially explain the positive result (392). The route of intrafamilial and sexual transmission is therefore undefined as yet. In chimpanzees, there is no evidence of nonparenteral transmission of HCV infection (359). Indirect data additionally suggest that nonparenteral transmission of HCV is different or less prevalent than nonparenteral transmission of HBV infection (Table 3). Thus, anti-HCV antibodies were not detected in any outpatients with developmental disabilities, whereas 21% were positive for markers of HBV infection (203). Even more striking, no institutionalized Down's syndrome residents or residents with other developmental handicaps were anti-HCV positive (EIA-2) (52). Again, exposure to HBV was extremely common. Similar results were reported for institutionalized clients in the United Kingdom, with none of 102 patients having evidence of HCV infection, although there was HBV exposure in 17 (64). Furthermore, in three separate studies, none of 200 Japanese children (396), 1,000 Chinese children (196), and 2,111 Peruvian natives (142) had markers of HCV infection despite "normal" levels of HBV exposure. Additional epidemiological studies are necessary to solve the perplexing problem of transmission of HCV in the absence of overt parenteral exposure.

Vertical transmission of HCV, although uncommon, has been well documented (144, 269, 275, 365, 399). Chronic non-A, non-B hepatitis was observed in 2 of 13 live-born infants of 11 mothers with chronic non-A, non-B hepatitis (401), suggesting the possibility of mother-to-child transmission. In eight infants born to mothers positive for both anti-HCV and HCV RNA, HCV RNA was either intermittently positive or positive in all samples of blood from infants up to 12 months of age (365). HIV infection was present in three of the mothers but only one child. Anti-HCV antibody was initially detected in the infants but was absent from all samples of infants more than 5 months old, suggesting that the initial positive results reflected passive transfer of maternal antibody. Aminotransferase elevation was intermittent or absent in the infants. Thus, neither antibody status nor biochemical studies indicated infection with HCV. However, in additional studies assaying both anti-HCV positivity and HCV RNA, vertical transmission was not detected (0 of 47) (309, 317) or was infrequent (5 of 87, 6%) (184, 402). The demonstration that maternal transmission of HCV infection in Japan was correlated with viral titers of $\geq 10^6$ per ml (207, 275), with no cases of vertical transmission at titers of $< 10^6$ per ml (275), may explain the differences in transmission rates. In summary, vertical transmission of HCV infection is infrequent, particularly in the absence of HIV coinfection or another factor(s) associated with high viral titers.

ACUTE HEPATITIS

The spectrum of liver disease observed in patients demonstrating seropositivity for HCV infection covers the gamut from asymptomatic blood donors with normal liver function tests and no apparent sequelae through acute and chronic hepatitis to hepatocellular carcinoma and hepatic failure requiring liver transplantation. At any one time, the number of patients with acute hepatitis C is likely to be the smallest clinical group. However, since every one of these patients could become chronically infected, they may all represent new additions to the reservoir of infected persons. The incidence of acute non-A, non-B hepatitis in the United States, derived by the Centers for Disease Control from the Sentinel Counties Non-A, Non-B Hepatitis Studies, is 7.1 per 100,000, and ~80% have HCV markers (10–12).

Acute hepatitis C, presenting de novo for diagnosis and management, is clinically indistinguishable from other causes of viral hepatitis. Following a viral prodrome, nonspecific gastrointestinal symptoms of anorexia and nausea usually develop, abdominal pain may occur, and jaundice may be noted. Laboratory investigations reveal elevated aminotransferase and alkaline phosphatase levels and elevated bilirubin in most patients with clinical illness. Results of serologic assays exclude acute hepatitis A and B (immunoglobulin M [IgM] anti-hepatitis A virus antibody negative, hepatitis B surface antigen [HBsAg] negative, and IgM anti-hepatitis B core antibody negative), and a careful history and physical examination can aid in excluding drug hepatotoxicity, ischemic hepatitis, biliary tract disease, and previously silent chronic liver disease.

Diagnosis of Acute Infection

A positive diagnosis of acute hepatitis C can be made by documentation of seroconversion to anti-HCV positivity. Unfortunately, differentiation of acute from chronic HCV infection cannot be made by measuring IgM anti-HCV antibodies since these may never appear, may appear late, and may persist with chronic infection (421). When anti-HCV antibodies are positive at the time of presentation, one problem is that of differentiating newly acquired, acute infection from an exacerbation of underlying chronic infection. Injection drug users, in particular, and others with continuing parenteral risk factors may be chronically infected with HCV without overt disease, as the blood donors described above. Exposure to another parenterally transmitted agent, not yet identified, may then explain the acute hepatitic illness. Alternatively, patients with chronic hepatitis C may develop an acute hepatitic illness with reinfection. Such a response is suggested by human and animal studies (87, 156, 183). Viremia occurred in chimpanzees after inoculation of different strains of HCV or inoculation with the same strain of HCV after clearance of detectable virus from the circulation, indicating that protective immunity did not develop (87). Similarly, acute hepatitis developed in a patient chronically infected with one genotype when infection with another genotype occurred (156), and multiple episodes of acute hepatitis were observed in polytransfused thalassemic children reinfected with different HCV strains (183).

Some patients with HCV infection and clinically apparent acute hepatitis may not develop seropositivity as measured by current EIA-2 assays (12). Of 106 patients with acute hepatitis and evidence of HCV infection, 93 (88%) were anti-HCV positive by commercial assay. Every anti-HCV-positive patient who was tested (n = 21) had HCV RNA in serum by PCR amplification. An additional nine patients (8%) were anti-HCV negative when tested but had HCV RNA present as a marker of HCV (12). In four patients who were anti-HCV negative on commercial testing (one of these patients was HCV RNA negative; three were not tested), antibodies that recognized HCV antigen in liver were present (12). These antibodies presumably recognize structural or other determinants not present on the linear peptide antigens used in EIAs. They blocked the binding of fluorescently labeled human IgG that is reactive with HCV antigens in frozen liver biopsy specimens from experimentally infected chimpanzees (176). Similar blocking antibodies were also present in 19 of 19 anti-HCV-positive patients and 5 of 9 patients with HCV RNA but without anti-HCV reactivity. Thus, readily available tests were able to identify 88% of HCV-infected patients, whereas research assays were needed for the remaining 12%.

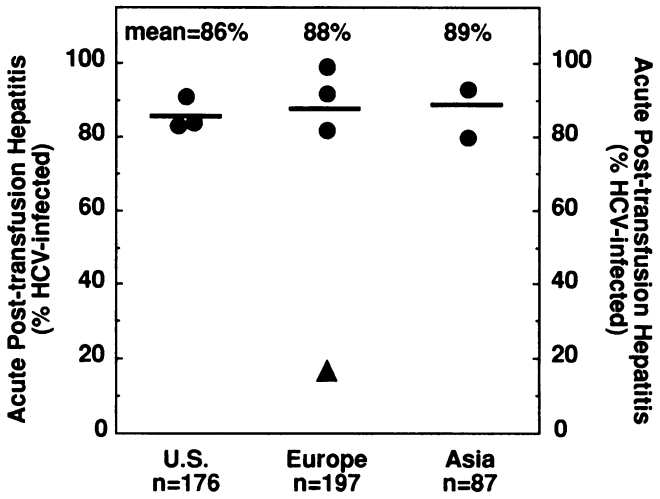

FIG. 5. HCV as a cause of acute posttransfusion hepatitis before (circles, United States, Europe, and Asia) and after (triangle, Europe) introduction of routine anti-HCV screening of blood donors. Data are from references 1, 12, and 173 (United States); 110, 235, 366, and 372 (Europe); and 217 and 393 (Asia).

HCV and Acute Non-A, Non-B Hepatitis

The proportion of acute non-A, non-B hepatitis patients exhibiting HCV markers varies with the assay(s) used, geographic origin of the patients, and the risk factor profile of the selected patients (12, 100, 110, 299). The highest rates were observed in Italy with the combination of EIA-2 and immunoblot assays plus HCV RNA detection (110, 299, 372). In Los Angeles patients with acute non-A, non-B hepatitis, anti-HCV positivity was higher in injection drug users (109 of 118, 92%) than in patients with "sporadic" infection (59 of 96, 61%) (100). HCV infection is usually demonstrated in ~90% of patients with posttransfusion hepatitis, but not 100%, even with the latest assays, as shown in Fig. 5. The extremely low rate in France (3 of 18 [17%] anti-HCV positive) may be explained in part by the introduction of anti-HCV screening during the prospective study (366). Control patients receiving autologous blood transfusions did not develop hepatitis (366), indicating that the heterologous blood transfusions received by the patients were the likely source, rather than another etiologic factor associated with their hospitalization. Thus, although HCV infection can now be detected, there are still some unresolved questions concerning the etiology of acute non-A, non-B hepatitis. Additional evidence suggests that there may be another parenterally transmitted agent that

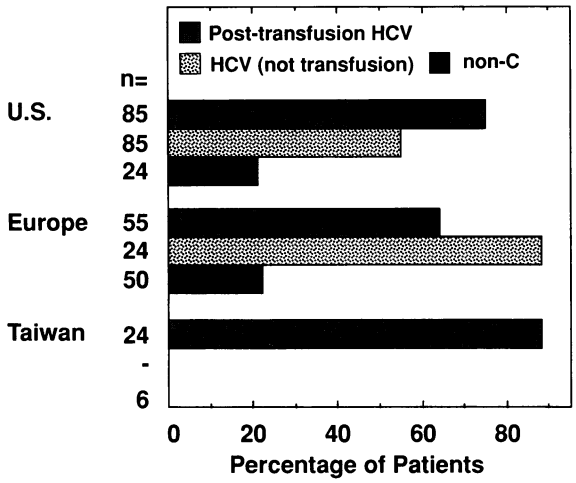

FIG. 6. Incidence of chronic hepatitis following acute non-A, non-B hepatitis. Data are from references 12 and 173 (United States); 110 and 235 (Europe); and 393 (Taiwan).

causes acute hepatitis (30). Both chloroform-sensitive (HCV) and chloroform-resistant (non-C) infectious agents transmitted hepatitis to chimpanzees (30). Another transmissible agent may therefore account for cases of acute non-A, non-B, non-C hepatitis with parenteral risk factors.

HCV and Fulminant Hepatitis

Fulminant hepatitis does not appear to be caused by HCV alone in most instances in the United States and Europe (91, 205, 322, 409). HCV RNA could not be amplified from serum and/or hepatic tissues of patients with fulminant acute non-A, non-B hepatitis (91, 322, 409). In contrast, HCV alone or coinfection of HCV together with other hepatitis viruses was observed in patients with fulminant hepatitis in Asia (61, 263, 410, 414). Occasional cases of fulminant hepatitis in patients positive for anti-HCV antibodies have been reported from Europe and the United States, but they are rare (177, 205, 330). An exception is a recent preliminary report from the United States of HCV RNA detection in 9 of 15 (57%) patients with fulminant hepatitis of indeterminate etiology, of whom four were anti-HCV positive (389). HCV RNA was also detected in two of seven patients with fulminant hepatitis B. HCV infection in this group of patients was associated with low socioeconomic level, and no patients had a history of parenteral risk factors (388). Until a definitive test for recent or acute HCV infection is devised, the possibility remains that these patients were chronically infected with HCV and then developed fulminant hepatic failure from another etiologic agent. The data thus suggest that both sporadic and parenterally transmitted acute infection may be caused by an agent other than those already identified.

Symptoms gradually resolve in the majority of patients with acute HCV infection. Laboratory investigations, however, may be persistently abnormal for months or years. The percentage of patients with documented chronic hepatitis following acute hepatitis C is usually very high (64 to 88%; Fig. 6) (12, 110, 173, 235, 393). Chronic hepatitis following acute posttransfusion hepatitis is no more common than that following sporadic or community-acquired disease when anti-HCV-positive cases are compared (12, 110, 173, 235). When anti-HCV tests are nonreactive, chronic hepatitis is less common than when these tests are positive (Fig. 6).

Whether HCV infection is eradicated and/or inactivated in the 12 to 36% of patients with clinical resolution of acute hepatitis is undetermined. Of great concern, HCV RNA was detected in all 15 patients (intermittently in 2) with normal aminotransferase levels and clinically resolved hepatitis who were tested in the Sentinel Counties study 42 to 48 months after their acute illness (12). Similarly, persistent viremia following biochemical resolution was observed in 10 patients with acute posttransfusion hepatitis (287). In other studies, however, clinical and biochemical resolution was accompanied by clearance of the virus from serum in all but one of a total of 25 patients (111, 133, 299). These data suggest that HCV infection may persist in some, but not all, patients when there is no evidence of clinical or biochemical disease. Persistent HCV RNA detection in serum, despite normalization of aminotransferase elevation, has also been observed following treatment with alpha interferon (152, 190, 325). Whether HCV infection is actually eliminated in patients with undetectable HCV RNA in serum remains unanswered.

CHRONIC HEPATITIS

Evidence of HCV infection is found in 60 to 100% of patients with chronic hepatitis when other causes are excluded and EIA-2 screening tests are used (38, 118, 148, 260, 311). Higher rates of HCV infection are observed in Japan and Mediterranean countries, and lower rates are seen in the United States and the United Kingdom. Measurement of HCV RNA by PCR does not substantially increase the number of patients demonstrated to have HCV infection. Thus, detection of HCV RNA in patients with chronic hepatitis, yet negative for anti-HCV antibodies by EIA-2 assays, is apparently uncommon (except in the immunosuppressed population), although there are few data to evaluate (55, 192, 260, 418). In French alcoholics, HCV RNA was detected in one of five patients negative for anti-HCV antibodies (192); however, no clinical data regarding chronic hepatitis or possible immunosuppression from coinfection with HIV were provided. Of four Japanese patients negative for both anti-C100-3 and anti-HCV core (C22) who had chronic non-A, non-B liver disease (one with chronic hepatitis, one with cirrhosis, and two with cirrhosis and hepatocellular carcinoma), only one with chronic hepatitis was positive for HCV RNA by PCR (418). Similarly, two Japanese patients (one with chronic hepatitis and one with cirrhosis) negative for anti-HCV antibodies (by EIA-2) were positive for HCV RNA by PCR (260). Different primer sets were required for one patient, indicating sequence variability. Anti-HCV antibodies recognizing antigenic epitopes not represented in current assays may be present in such patients, thereby accounting for the negative result on testing with second-generation assays. In contrast to these isolated patients, 156 (91%) of 172 consecutive Italian patients with chronic non-A, non-B hepatitis had anti-HCV antibodies whereas 9% (26 of 172) did not; 86% (89 of 104) of anti-HCV-positive serum samples were HCV RNA positive, but none of the anti-HCV-negative serum samples contained HCV RNA (55).

In patients with chronic hepatitis and anti-HCV antibodies by EIA-2, HCV RNA is very frequently detected (211, 260). In Hong Kong, 67 of 81 (83%) patients with anti-HCV antibodies (EIA-2) were viremic when two different primer sets (5′ noncoding and NS4) were used for PCR amplification (211). Similarly, 201 of 224 (90%) Dutch patients who were anti-HCV positive by EIA-2 and had multiantigen positivity on immunoblot were viremic; 21 of 31 (68%) with C22 or C33c single-antigen positivity on immunoblot ("indeterminate") were also viremic (35). In Japan, 98 of 100 patients with

chronic non-A, non-B liver disease were positive for anti-HCV antibodies by EIA-2 and 100 of 100 were positive for HCV RNA by PCR (260). Chronic HCV infection as an etiologic agent in chronic non-A, non-B liver disease can thus be readily identified in most patients with currently available second-generation assays.

HCV and Other Chronic Liver Diseases

The potential role of HCV infection in the chronicity or progression of liver diseases considered secondary to other etiologic agents has also been examined. The proportion of patients with (ostensibly) other liver diseases, but having evidence of HCV infection, is generally highly related to the country of origin of the patient group. Anti-HCV antibodies were positive in 15 of 88 Italian patients with primary biliary cirrhosis (22), for example, but were not detected in 31 British patients (239). Italian patients with hereditary hemochromatosis also commonly manifested evidence of HCV infection (10 of 78, 13% anti-HCV and HCV RNA positive) and chronic hepatitis on liver biopsy (288). The finding of anti-HCV antibodies in patients with alcoholic liver disease is also dependent on geographic origin (see below). In α1-antitrypsin deficiency, liver involvement is variable and HCV infection may account for development of overt hepatic manifestations (297). Among 53 patients with cirrhosis and either homozygous or heterozygous α1-antitrypsin deficiency, anti-HCV antibodies (EIA-2) were detected in 62% (33 of 53) (297). Markers of HCV infection were positive in only 4 of 78 (5%) patients with fatty liver, rather than cirrhosis, and either heterozygous or homozygous α1-antitrypsin deficiency. The data suggest that patients with significant liver disease and α1-antitrypsin deficiency may have another etiopathologic factor in addition to the abnormality in α1-antitrypsin.

Autoimmune Chronic Liver Disease

There is an especially complicated relationship between HCV infection and autoimmune chronic liver disease (reviewed in reference 306). In Italy, anti-HCV antibodies found in patients with autoimmune chronic hepatitis on first-generation testing were generally confirmed reactive on second-generation assays (46, 200, 220) regardless of the type of autoimmunity. Additionally, antibody reactivity was often associated with HCV RNA positivity in Italian patients with autoimmune features (10 of 15 [67%], using a single primer set) (220), indicating true infection. Japanese patients with autoimmune chronic hepatitis were also likely to be reactive on both first- and second-generation assays for anti-HCV antibodies, but HCV RNA was less often detected (5 of 26 [19%], using nested primers from the 5′ noncoding region) (266). In contrast, Swedish and British patients with autoimmune chronic hepatitis had false-positive anti-HCV reactivity that correlated with disease activity and hyperglobulinemia (200, 239, 332). Similarly, although serologic markers of HCV infection were found in 8 of 17 (47%) U.S. patients with autoimmune hepatitis, these were generally not confirmed by immunoblot (2 of 17 positive, 12%) or detection of HCV RNA (1 of 17, 6%) (101).

The association between HCV and type 2 autoimmune chronic liver disease suggests a relationship other than a false-positive anti-HCV test or coexistence of both diseases (306). In type 2 autoimmunity, antibodies to liver and kidney microsomes (anti-LKM) are present. Anti-LKM_1 recognizes cytochrome P-450IID6 (227), and patients with anti-LKM_1 antibodies are distinguishable from those with antibodies against liver and kidney microsomes in association with tirynafen-induced liver disease (anti-LKM_2) or infection with hepatitis B and D viruses (anti-LKM_3). Patients with anti-LKM_1 antibodies frequently have antibodies to HCV and HCV RNA detected (105, 199, 216, 247). This is particularly so in older patients, especially men, with low titers of anti-LKM_1 antibodies (216). Younger patients are more often negative for anti-HCV antibodies (200, 216). Additionally, patients with HCV infection and anti-LKM_1 antibodies are also frequently positive for antibodies directed against a cross-reactive determinant expressed in liver, GOR. GOR antigen shares part of its amino acid sequence with the HCV core protein (136, 423). Anti-GOR antibodies were found in 10 of 31 (32%) U.S. patients with chronic hepatitis C (189). The presence of anti-GOR has no clinical or prognostic significance in patients with chronic hepatitis C (189). The development of other autoantibodies in association with HCV infection appears to be confined to a minority of patients in a limited geographic distribution. HCV genome analysis in German and Italian patients with (n = 43) and without (n = 82) anti-LKM_1 antibodies did not find an association of antibodies with a specific HCV genotype (248). The geographic distribution may therefore reflect differences in the major histocompatibility complex and the selection of specific peptides for presentation to autoreactive lymphocytes.

Alcoholic Liver Disease

Early studies of U.S. patients with alcoholism demonstrated an increased frequency of anti-HCV positivity (27% by EIA-1) in those with clinical liver disease (245). There was no such relationship for antibodies to hepatitis B. However, many of the reactive sera were negative by confirmatory immunoblot. The reported decreased survival in those patients with unconfirmed reactivity was unexplained (245). Antibodies to HCV, found in up to 68% of selected Japanese patients with alcoholic liver disease, were associated with HCV RNA positivity and increased histopathologic damage (267). Similar findings were made in a Spanish investigation (283). In France, 22 of 62 (35%) patients with chronic alcoholism had anti-HCV antibodies (second-generation immunoblot) and HCV RNA was also detected in 18 of the 22 patients (262). Liver biopsies demonstrated only alcoholic liver disease in seven patients with viremia, but a mixed picture of virus- and alcohol-related pathologic lesions was observed in five patients. Infection with HCV may substantially increase the likelihood of significant liver disease in alcoholic patients. The finding that anti-HCV positivity in alcoholic patients could often be explained by a history of previous injection drug use (97, 314, 385) suggests the coexistence of two relatively common potential causes of chronic liver disease. Whether there is an interaction between the pathogenetic processes, however, is less clear.

Hepatitis B Coinfection

Because of shared epidemiology, coinfection with both HBV and HCV occurs. Evidence of coinfection with both B and C hepatitis viruses was observed in 16 of 148 (11%) patients monitored at the National Institutes of Health (96). These patients were more likely to have cirrhosis and decompensated liver disease and have HIV infection, consistent with shared epidemiology. Retrospective analysis of patients prospectively monitored for posttransfusion hepatitis demonstrated that of 12 patients who developed hepatitis B, 5 were infected with HCV concurrently (249). Coinfection with both viruses delayed the appearance of HBsAg, reduced the antigen concentration, and was associated with lower aminotransferase levels and a biphasic pattern of elevation. In Chinese patients with

chronic hepatitis B who underwent spontaneous clearance of HBsAg, markers of HCV infection (EIA-2) were found much more frequently (17 of 54, 31%) than in matched controls (9 of 152, 6%) who remained HBsAg positive (337). Other investigators monitoring small numbers of patients have not observed any significant interaction between chronic HBV infection and HCV (393).

A potential explanation for a decrease in HBsAg has been suggested by recent experimental studies (341). Expression of HCV core gene sequences in a human hepatoma cell line interfered with and suppressed replication of hepatitis B and secretion of hepatitis B viral particles (341). Furthermore, a reciprocal inverse pattern of viral interference was observed in 25 Italian patients chronically coinfected with both HCV and HBV (292). When serum contained sufficient HBV DNA to be detected without PCR amplification (10 patients), HCV RNA was positive in only 1 patient (10%) whereas if HBV DNA was undetectable in serum (15 patients), 13 patients (87%) were HCV RNA positive. Similarly, HCV RNA was not detected in any of five anti-HCV-positive hemophiliac patients who were also positive for HBsAg, whereas 33 of 37 patients without HBV coinfection had detectable HCV RNA (126). Whether viral interaction explains these findings is uncertain; experimental studies may provide answers in the future.

CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

In many patients, HCV infection results in progressive damage and the development of cirrhosis and its complications. However, patients with progressive liver disease may not be symptomatic, even when cirrhosis has developed. Furthermore, overall survival may not be altered by HCV infection acquired from transfusion (334). Thus, in a follow-up study of patients prospectively diagnosed as having posttransfusion non-A, non-B hepatitis, survival was the same as in controls. There was a modest increase in liver disease-related mortality (total 3.3%; compared with 1.1 and 2% in two separate control populations). Alcohol was potentially a cofactor in this increase (334). These data suggest that, in the majority of patients, HCV infection follows an indolent course. Similarly, when patients receiving organ transplants from anti-HCV-positive donors were monitored clinically, few developed significant liver disease (315). In contrast, a subgroup of patients clearly had progressive disease and developed complications of chronic liver disease and hepatocellular carcinoma.

At the time of diagnosis, patients with chronic hepatitis C (abnormal aminotransferase levels during a 6- to 12-month period of observation and anti-HCV antibody positive) may have any of a range of abnormalities on liver biopsy (Fig. 7). Thus, liver biopsy demonstrated chronic hepatitis in 50 to 90% and cirrhosis was present in 10 to 55% at the time of presentation with chronic hepatitis C infection (67, 71, 246, 260). Longitudinal studies of patients with posttransfusion chronic disease indicate that chronic hepatitis is present on biopsy an average of 10 years after transfusion (range, 1 to 45 years), and cirrhosis is present by 20 years after (range, 10 to 50 years) (164). However, cirrhosis can be present in <2 years in individual patients with particularly aggressive disease (71), and other investigators have reported that histological severity does not correlate with duration of disease but does correlate with age (246). Either viral or host factors, or a combination of these, may be important in determining outcome. In a study of 251 Japanese patients, neither genotype nor viral titer was associated with disease status (411). If viral factors are not important in determining progression, then histological severity may be entirely dependent on the host response to the chronic infection.

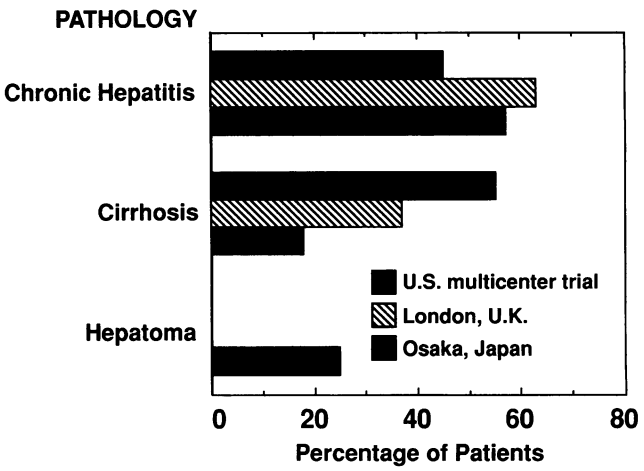

FIG. 7. Histopathologic diagnosis in patients with chronic liver disease from HCV infection. Data are from references 67, 118, and 246.

In renal transplant recipients, chronic infection with HCV is common, being found in 28 of 100 U.S. patients (187), 22 of 120 Belgian patients (113), and 23 of 151 (12%) patients in Hong Kong (50). An indolent or clinically silent course appears to be the usual finding (113, 187, 312, 316), suggesting that chronic immunosuppression may decrease progression. However, poor outcome is observed in some patients (187), and the length of follow-up in others (generally <10 years) may be insufficient for the development of complications from chronic liver disease. Whereas overall patient survival is unaffected by HCV infection (103, 187, 316), reduced graft survival was noted in anti-HCV-positive black renal transplant patients with HCV infection in one study (103) and increased risk of infection and rejection were seen in another study (316). Whether HCV will remain clinically silent in these immunosuppressed transplant recipients is yet to be determined.

HCV infection is associated with the development of hepatocellular carcinoma, as reported by a number of investigators (194, 355, 375, 422; reviewed in reference 310). As noted above, in patients with posttransfusion hepatitis monitored long term, hepatocellular carcinoma is a late occurrence, taking ~30 years (range, 15 to 60 years) to develop (164). At the time of initial presentation, hepatoma was infrequent in the United States and Europe but was found in up to 30% of Japanese patients (118, 260). In a review of reports that determined the incidence of viral markers in 1,930 patients with hepatoma, HCV infection was present in 501 patients (26%) without HBV markers, in 221 patients (11%) with antibodies to hepatitis B viral markers, and in 177 patients (9%) with ongoing hepatitis B infection (310). Thus, HCV infection potentially contributed to the development of hepatoma in 47% of the patients. Since neither second-generation assays nor HCV RNA was measured in every study, this is likely to be an underestimate of the importance of HCV in the development of hepatoma. A study of 91 U.S. patients with hepatocellular carcinoma, all negative for HBsAg, found serological or PCR positivity indicating HCV infection in 53 patients (58%), 14 of whom had evidence suggesting occult coinfection with HBV (204). A lower incidence of HCV infection (14 of 87, 16%) was reported in another U.S. study (226). Variation in incidence may reflect differences in viral,

host, or environmental factors. Thus, hepatoma complicates chronic HCV infection, and HCV is the second most common etiologic agent in the development of this disease.

HCV AND OTHER DISEASES

Essential mixed cryoglobulinemia is strongly associated with HCV infection (3, 94, 95, 251; reviewed in reference 202). Recent case series have detected HCV RNA and anti-HCV antibodies in the majority of patients with type II and type III mixed cryoglobulinemia, suggesting that circulating HCV antigen and antibody may induce the disease in susceptible individuals. Purpura improved in patients during treatment with alpha interferon, but symptoms rebounded at completion of the course of therapy (95, 252). Membranoproliferative glomerulonephritis is also associated with HCV infection (149). Both liver and renal disease improved in four patients with decreased HCV replication following alpha interferon therapy. A treatment regimen that results in sustained resolution of HCV viremia or antigenemia is likely to improve both essential mixed cryoglobulinemia and membranoproliferative glomerulonephritis. Porphyria cutanea tarda, a disease characterized by cutaneous lesions and commonly associated with liver disease, appears to become clinically manifest when extrinsic factors that cause liver disease, such as HCV infection, are present (70, 89, 129, 182). In Italy, ~80% of 74 patients had markers of HCV infection by EIA-2 and immunoblot assay (89). In contrast, aplastic anemia following non-A, non-B hepatitis is not associated with HCV infection (79, 130, 289, 291) except rarely (116).

PATHOLOGY

Liver biopsy findings in patients infected with HCV necessarily range from mild, nonspecific changes through chronic hepatitis with varying amounts of portal and/or lobular inflammation and fibrosis to cirrhosis and hepatocellular carcinoma (328). The previous practice of subdividing chronic hepatitis into chronic persistent and chronic active hepatitis is less useful in HCV infection than HBV infection (214). None of the findings are pathognomonic for HCV infection, but some of the histological appearances are found more commonly with hepatitis C than in hepatitis due to other etiologic agents (17, 66, 197). Direct comparison of hepatitis C with autoimmune chronic hepatitis has revealed histological features that are more often seen in one or the other disease (17). The more indolent nature of hepatitis C in many patients, with relatively less aggressive disease and a lower incidence of cirrhosis, was apparent in the comparison. A histological pattern of mild chronic hepatitis with portal lymphoid follicles, mild fatty infiltration, and loss of bile ducts was the most common finding with HCV infection (17). Three features more likely to be seen in HCV infection than in hepatitis B were identified by comparing biopsies from patients entered into two large treatment trials (197). These were bile duct damage, lymphoid follicles, and large droplet fat. In addition, Mallory body-like material was seen only in HCV infection and not in HBV infection (197). Specific patterns, while helpful in some patients, are neither unique nor universally present (66).

HCV-infected cells in human liver have been identified immunohistochemically by using antibodies to the core, envelope, NS3, NS4, and NS5 regions of the HCV genome (26, 134, 323, 376). Hepatocyte cytoplasmic staining was detected in liver tissue from 19 of 48 patients with core, envelope, and NS3 region antibodies (134). Inflammation and fibrosis scores were higher in positive tissue. The HCV C100-3 antigen has also been detected in fresh frozen liver tissue from patients with acute and chronic infection, using indirect immunofluorescence (323). In acute hepatitis, the majority of hepatocytes (50 to 70%) were reactive, whereas very low numbers of cells were positive in chronic hepatitis. In contrast, only reactivity to an antigen(s) encoded by the NS4 region was observed in formalin-fixed tissue by other investigators (26). Most hepatocytes (60 to 90%) appeared positive, with variable intensity of the staining pattern. Antigens encoded by the E2, NS3, and NS5 regions were not detected (26). The precise relationship between the expression of various viral antigens in hepatocytes and the progression of liver disease has not been established.

TREATMENT

Recombinant alpha interferon was approved for treatment of chronic hepatitis C following trials demonstrating sustained normalization of aminotransferase levels in ~25% of U.S. patients after treatment with 3×10^6 U thrice weekly for 24 weeks (67, 73). Beta interferon has also been tested for efficacy, particularly in Japan. An increase in sustained response rates to alpha interferon therapy may be achieved by increasing the treatment time from 24 weeks to 48 or 60 weeks (151, 209, 307, 325). With an alternative regimen of 10×10^6 U six times a week for 2 weeks followed by three times a week for 12 weeks, sustained responses were observed in 48% (14 of 29) of Japanese patients (143). However, besides the dose regimen, differences in the treated population compared with patients from other areas may account for the increased rate of response, since this magnitude of increase has not been observed by other investigators (209). Improvement in pathology has been observed in some studies (224, 325) and, with regimens having improved efficacy, may be found more often in the future.

A number of investigators have reported that serum HCV RNA became undetectable or titers diminished in the patients responding to alpha interferon therapy (37, 53, 121, 221, 345, 347). During relapse, serum HCV RNA once again emerged. Long-term follow-up has confirmed these findings in patients initially entered in pilot studies (347). However, there are many exceptions to these observations, with patients achieving sustained normalization of hepatic aminotransferase levels despite re-emergence or persistence of serum HCV RNA (152, 190, 325) or relapsing despite continued loss of serum HCV RNA (170). Measurement of HCV RNA in liver after therapy generally correlates with initial clinical outcome, with disappearance in all (109) or most (18) patients achieving sustained responses. HCV RNA in liver or serum can also decrease significantly without improvement in elevated aminotransferase levels (206). Concomitantly with decreases in hepatic HCV RNA, HCV antigen detected by immunofluorescence staining may decrease in patients responding to interferon therapy (72). Circulating anti-HCV antibodies may also fall in response to interferon therapy. Thus, antibody to C100-3 can become undetectable in responders during follow-up (325, 417). In addition, titers of IgM anti-HCV core antibodies may reflect disease activity (258). Thus far, however, there are no absolute measurements that can predict sustained responses.

Natural beta interferon has been tested in short-course therapy (intravenously for 4 weeks) for hepatitis C and non-A, non-B hepatitis in Japan (274, 278). In an early pilot study, five of five patients with chronic posttransfusion non-A, non-B hepatitis responded initially, with decreases in aminotransferase levels, but there were no sustained responses (274). Of six patients diagnosed as having acute posttransfusion non-A, non-B hepatitis, five patients responded with decreases in

aminotransferase levels, and at 1 year follow-up, all six patients had normal aminotransferase levels (274). A second study randomized patients with acute non-A, non-B hepatitis to treatment with beta interferon or no therapy (278). HCV RNA was detectable in 10 of 11 treated patients and 12 of 14 controls initially. After a single 4-week course of therapy, seven patients responded with sustained normalization of aminotransferase levels and three of four patients, who initially were nonresponders and were retreated after 1 year, also responded. In the control group, only three patients spontaneously normalized aminotransferase levels, between 10 months and 2.5 years after onset. These results, while encouraging, are preliminary. Additional studies are needed to determine if beta interferon is the optimal treatment for acute hepatitis C.

Predicting Sustained Responses

Host and viral factors that predict likelihood of a sustained response to interferon therapy have been analyzed in a number of studies. Patients with advanced disease are less likely to respond (45, 151, 281, 325, 349), as well as being poorly able to tolerate side effects. Obesity, elevated gamma glutamyl transpeptidase levels, and older age may be markers of poor response (6, 45, 236). Response rates to alpha interferon therapy may also be adversely affected by increased hepatic iron (384). The effect of iron depletion on response rates to interferon therapy is being evaluated.

The possibility that HCV genomic type and/or viral titer may influence the likelihood of achieving remission has been suggested. Patients with HCV genomes similar to the prototype U.S. strain (type 1 HCV, Simmonds classification in references 351 and 352) had higher virus concentrations and lower complete response rates (5 of 39 [13%] complete responses) than patients with strains more commonly isolated in Japan (type 2 HCV; 16 of 26 [62%] complete responses) (415). Similar results were obtained in other studies of Japanese patients infected with type 1 or type 2 HCV who were treated with natural alpha interferon (253, 374), alpha interferon subtype 2a (234), and alpha interferon subtype 2b (132). Lower titers of HCV RNA have been correlated with greater likelihood of a sustained response to natural alpha interferon (120, 253), alpha interferon subtype 2b (132, 188, 222), and beta interferon (171). Pretreatment viral titer was more important in predicting response to beta interferon than was genotype (171). In a group of six patients with chronic hepatitis C, there was heterogeneity in the hypervariable region sequence from the three individuals that did not respond to beta interferon therapy (276). In contrast, the three patients who demonstrated a sustained response and clearance of HCV RNA from the circulation had little or no heterogeneity in the hypervariable region. Furthermore, in a small study of patients treated with alpha interferon, the mutation rate in the hypervariable region was higher in nonresponders (342). Future evaluation of HCV-infected patients may include genotyping of the viral strain and assessment of homogeneity and mutation rates before selection for particular treatment protocols.

Patient Selection

Selection of patients with chronic hepatitis C for interferon therapy requires care and consideration. In occasional patients with features of autoimmunity, liver disease is exacerbated by alpha interferon (282, 346). Occult, autoimmunity-related thyroid disease (229) and thrombocytopenia can become symptomatic, and severe exacerbation of lichen planus has been reported (298). The observation of a high prevalence of thyroid autoantibodies (9 of 29 patients, 31%) in females with hepatitis C before interferon therapy and hypothyroidism in two patients before treatment and three other patients after treatment (371) may explain the occurrence of overt thyroid disease.

Before embarking on an expensive course of therapy that may only benefit a minority, additional consideration should be given to particular groups of patients, including those with recent active substance abuse, alcoholic liver disease, immunosuppression associated with chronic renal insufficiency, coinfection with HIV or organ transplantation, psychiatric disease, and significant cardiac disease (406). Treating patients that are most likely to respond and benefit from therapy is the goal. Those with persistently or intermittently abnormal serum aminotransferase levels and progressive disease (development of increased fibrosis) on liver biopsy are candidates for therapy. Decisions regarding patients with inflammation but no fibrosis are more difficult. Such patients can be monitored and observed without treatment or can be offered a course of therapy (406).

Therapy for Acute HCV Infection

Randomized studies have also assessed the efficacy of alpha interferon therapy in acute posttransfusion hepatitis C (185, 386). Aminotransferase levels were normal in a mean of 66% (11 of 15 and 16 of 26) of treated patients in two separate studies but in only 38% (5 of 13 and 7 of 19) of controls after 3 months. In one study, there were no significant differences between the groups by 12 months follow-up, although more treated patients had normal aminotransferase levels (8 of 15 [53%] compared with 4 of 13 [31%] in the control group) (386). In the second study, the results were similar: 13 treated patients (59%) and 6 controls (37%) had normal aminotransferase levels at 18-month follow-up (185). However, HCV RNA was absent and aminotransferase levels were normal in seven treated patients (39%) but in none of the controls, suggesting the possibility of long-term quiescence. Additional studies of therapeutic regimens for acute disease are clearly needed.

Newer antiviral agents and combination therapies are undergoing testing. Ribavirin therapy decreased HCV RNA titers and normalized serum aminotransferase levels in treated patients; however, the response was not sustained (74, 308). Ribavirin in combination with beta interferon has also been tested in pilot studies: unfortunately, without dramatic improvement in response rates (153). In another preliminary study, alpha interferon in combined therapy with oral *N*-acetylcysteine improved aminotransferase levels in individuals previously unresponsive to alpha interferon alone (21); however, HCV RNA persisted in most patients and long-term follow-up was not reported. Regimens that combine interferon therapy with corticosteroids, ursodeoxycholic acid, or depletion of hepatic iron by phlebotomy are under investigation. Until better agents are available, alpha interferon is the mainstay of therapy. Trials examining longer duration of therapy and alteration in dosage may provide a regimen with somewhat higher predicted response rates.

TRANSPLANTATION

In a subset of HCV-infected patients, continuing hepatocyte infection leads to progressive liver disease, the development of cirrhosis, and potentially life-threatening complications. The only currently available therapy for such patients is liver transplantation. Patients with progressive HCV infection constitute a significant proportion of liver transplant candidates.

TABLE 4. Evidence of histologic hepatitis in liver transplant recipients with hepatitis C infection[a]

Posttransplant HCV RNA in serum	No. (%) of patients with posttransplant hepatitis	
	Definite/probable	Absent
Positive (n = 55)	28 (51)	27 (49)
Negative (n = 60)	3 (5)	57 (95)

[a] Data from references 215 and 408.

Of 128 transplant recipients at the University of California, San Francisco, 20% were HCV infected pretransplant: 15 of 30 with chronic non-A, non-B liver disease, 7 of 19 with a diagnosis of alcoholic cirrhosis, and 3 of 11 with HBV-related liver disease were positive for anti-HCV antibodies (EIA-1 and neutralization confirmation) (305). No patients with other causes of chronic liver disease were reactive. Overall results were similar at the University of Pittsburgh: 105 of 372 (28%) transplanted patients were anti-HCV seropositive (81). However, patients with diagnoses other than chronic viral liver disease and alcoholic cirrhosis were also positive for anti-HCV antibodies. Indeed, there was little difference in the seropositive rate in the various disease categories, suggesting the possibility of artifact or that hepatitis C contributed to the progression of nonviral liver diseases. Anti-HCV seropositivity in liver transplant candidates was lower (34 of 317, 11%) in later studies from the same institution (336). Without HCV RNA measurements, however, the true rate of HCV infection cannot be determined precisely.

The problem of recurrence of hepatitis C posttransplant is one of great concern (215, 407). HCV infection posttransplant can be newly acquired or a recurrent infection. When initially evaluated, the rate of HCV infection in liver transplant recipients was underestimated because few patients seroconverted for anti-HCV antibodies posttransplant and some lost preexisting antibody with immunosuppression (305, 408). Studies using PCR to identify patients with HCV infection pretransplant and posttransplant have allowed better estimates of infection rates (172, 293, 408). In 89 U.S. liver transplant patients with alcoholic or cryptogenic cirrhosis, HCV RNA was detected in 30 of 35 anti-HCV-positive patients and in 6 of 54 patients negative for anti-HCV (408). In the posttransplant period, all of the patients with anti-HCV reactivity were viremic. In addition, four of six patients with detectable HCV RNA pretransplant but no anti-HCV antibodies were also viremic posttransplant. Another 17 patients had HCV RNA first detected posttransplant (408). With better screening of blood and blood products, HCV infection acquired following liver transplantation should be less common in the future.

Whether immunosuppression also alters the relationship between the virus and host is less clear. Although viral titers are higher after liver transplantation (54), this does not always lead to hepatocyte damage. In studies from the United States (408) and Spain (215), posttransplant hepatitis in the allograft was diagnosed in a mean of 51% of the patients with HCV RNA and was rare without evidence of HCV infection (Table 4). Of note, however, a hepatitic pattern was not seen on liver biopsy in 49% of these immunosuppressed, viremic patients with HCV. In another study, HCV infection was detected by PCR in 15 of 25 (60%) patients with chronic hepatitis in an allograft; only 7 of the 15 (47%) patients were positive for anti-HCV antibodies (293). Not all transplant programs have observed a high rate of persistent HCV infection after liver transplantation. Thus, HCV RNA was detected posttransplant in only 4 of 14 (29%) French patients who were previously anti-HCV positive (92). Why the rate of persistent infection was so low in this group of patients, compared with others, has not been explained. In summary, hepatitis C after liver transplantation, as either a recurrence or a new infection, may be a frequent cause of chronic hepatitis and difficult to diagnose by conventional methods. However, the presence of persistent HCV infection posttransplant does not necessarily result in recurrent hepatitis. Long-term follow-up studies are needed to determine the importance of hepatitis C in the survival of liver transplant recipients.

PREVENTION OF INFECTION

By screening the blood supply (19, 77, 147) and by inactivating undetected virus (270), it is hoped that future transmission of HCV by blood and blood products may be eliminated. Even products with currently low rates of hepatitis transmission, such as intravenous immune globulin, need to be monitored (377). Introduction of tests for anti-HCV antibody detection has resulted in a decrease in the incidence of posttransfusion hepatitis (19, 77, 147, 361). Furthermore, the low rate of posttransfusion hepatitis in countries like the United Kingdom and Sweden can be further decreased by the use of anti-HCV screening, without discarding too high a percentage of donations (63, 232). Improvements in blood donor screening by direct questioning about medical history and social behavior have also contributed to the decrease in posttransfusion hepatitis (25).

Postexposure prophylaxis recommendations for needle stick transmission are not uniform. Intramuscular immune serum globulin is prescribed by some (75, 108) since studies, including recent investigations (7), have shown statistical benefit. The prospects for true prevention, vaccine development, have been gloomy. However, preliminary reports suggest that immunization of chimpanzees with glycosylated E1 and E2 complexes may provide protective immunity (58). Thus, five of seven chimpanzees vaccinated with complexes from type 1 HCV (Simmonds classification) demonstrated a strong humoral immune response and were protected from infection with homologous virus. Whether such an approach can be successfully employed to prevent infection from multiple viral genotypes is not yet known. Alternatively, injection of plasmids that express HCV proteins in vivo may lead to the development of protective cytotoxic T-lymphocyte responses (238), as reported for influenza (378). The lack of evidence for sustained clearance of the virus in the majority of humans or development of immunity to cross-challenge makes the task of vaccine formulation daunting.

CONCLUSIONS

HCV, the major cause of posttransfusion hepatitis, is a single-stranded RNA virus with sequence similarities to the *Flavivirus* and *Pestivirus* genera of the family *Flaviviridae*. Like other RNA viruses, HCV exists in a quasispecies distribution with different nucleotide and amino acid sequences in isolates of HCV obtained from a single individual as well as from different individuals. Nucleotide changes between different strains of the virus are concentrated in hypervariable regions and may be related to immune selection. In most immunocompetent persons, HCV infection is detected by assays using recombinant antigens from conserved core and nonstructural (NS3 and NS4) regions. Amplification of RNA from the serum or liver, by PCR with oligonucleotide primers from highly conserved areas of the 5′ noncoding region, may be necessary

to detect infection in immunosuppressed patients or those with very low viral titers. Transmission of HCV by the parenteral or percutaneous route is frequent; spread by sexual contact or vertical transmission is much less common and may result from inapparent percutaneous inoculation with high viral titer fluid. Infection with HCV can lead to classical acute hepatitis, but most anti-HCV antibody-positive subjects have no history of acute disease. Many infected people remain carriers of the virus, with varying degrees of hepatocyte damage and fibrosis ensuing. Chronic hepatitis may progress to cirrhosis; however, time intervals vary from less than 2 years to more than 40 years. Hepatocellular carcinoma may also ensue, generally after at least 10 years of infection in those with progressive disease. Host determinants currently appear more important than viral determinants in deciding outcome. Viremia and antigenemia from HCV infection are also associated with essential mixed cryoglobulinemia and membranoproliferative glomerulonephritis from antigen-antibody interaction. Currently, alpha interferon, which results in sustained remission of aminotransferase elevation in selected patients, is the only available therapy. HCV genotypes and viral titers may be associated with different therapeutic responses. HCV infection, alone or as a cofactor with other agents, accounts for a significant number of patients requiring liver transplantation for complications of cirrhosis. Following transplantation, HCV infection recurs in the transplanted liver but is generally not aggressive in nature. Prevention of HCV infection by vaccination is challenging because of ongoing viral mutation; however, immunization with native envelope proteins or in vivo expression of viral epitopes may be efficacious.

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