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have a more stabilizing effect on the IRE-BP than direct iron starvation or administration. Finally, treatment of RD4 cells with puromycin at doses that completely inhibit protein synthesis has no effect on the induction of IRE-binding activity in response to Df (*19*).

We therefore propose that chelation of iron by Df results in the activation of the IRE-BP by leading to the reduction of an intramolecular disulfide in the IRE-BP. At least one of the now free cysteinyl residues is required for a high affinity interaction between the protein and the IRE which, in turn, is responsible for the repression of ferritin mRNA translation. In essence, alteration in cellular iron status operates a "sulfhydryl switch" by reversible oxidation or reduction of critical sulfhydryl group or groups in the IRE-BP. This hypothesis raises the question of whether such a switch could be physiologically relevant in the reducing environment of the cytosol. The major redox buffer in the cytosol is the glutathione system. The vast excess of reduced over oxidized glutathione is largely responsible for the reducing potential of the cytosol. A study on the reversible oxidation-reduction of 3-hydroxy-3-methylglutaryl coenzyme A reductase demonstrates that oxidized sulfhydryls can exist and even predominate within the cytosolic glutathione redox buffer system (*23*). Two factors can determine the redox state of a protein sulfhydryl within the cytosol. One is the ratio of reduced to oxidized glutathione, which can change significantly under physiologic conditions (*23*). The second is the oxidation equilibrium constant (K_{ox}) for a particular sulfhydryl group within a protein. Equilibrium constants for protein sulfhydryls can vary over many orders of magnitude, reflecting the effects of the local environment around the cysteinyl moiety on its K_{ox} (*24*). These local effects may reflect the stabilization or destabilization of the thiolate anion. Conformational changes that alter this local environment can therefore affect the K_{ox} of a particular cysteine sulfhydryl group. In this way allosteric effectors can perturb the K_{ox} of sulfhydryls on specific proteins and thereby alter the redox state of the protein, even in the presence of a constant cytosolic redox buffer. Our data on the IRE-BP provide an example of the utility of oxidation-reduction as a reversible covalent modification in the regulation of cellular protein function.

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17. Partial purification of the IRE-binding protein was achieved by RNA affinity chromatography. An IRE-containing RNA was transcribed in vitro by RNA polymerase T7 (*18*) with two synthetic oligodeoxynucleotides that when hybridized constituted an RNA polymerase T7 promoter and a template for the ferritin IRE (Fig. 1) plus the 3' nucleotides $UCUCUCUCU_{34}$. The reaction included 3 m*M* biotin-11–uridine triphosphate (UTP) (Bethesda Research Laboratories) and 1 m*M* UTP, in addition to [α-^{32}P]guanosine triphosphate. The RNA was purified by hybridization chromatography on poly(A) Sepharose (Pharmacia). RNA was added to a lysate of K562 cells and incubated for 30 min at room temperature before addition of 5 mg of sodium heparin (Ilynson, Westcott, and Dunning) per milliliter. This sample was then added to streptavidin agarose (Bethesda Research Laboratories) and the mixture was incubated for an additional 15 min before the resin was washed five times with 20 volumes of 40 m*M* KCl, 25 m*M* tris-Cl, *p*H 8, and 1% Triton X-100. Elution of IRE-BP was accomplished with 1*M* KCl, 5 mg of sodium heparin per milliliter, 25 m*M* tris-Cl, *p*H 8, and 1% Triton X-100. Based on recovery of IRE-binding activity and the recovery of labeled K562 protein from a parallel experiment with a [^{35}S]methionine lysate, we estimate that the IRE-binding protein was purified approximately 60-fold by this procedure.
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Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome

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A random-primed complementary DNA library was constructed from plasma containing the uncharacterized non-A, non-B hepatitis (NANBH) agent and screened with serum from a patient diagnosed with NANBH. A complementary DNA clone was isolated that was shown to encode an antigen associated specifically with NANBH infections. This clone is not derived from host DNA but from an RNA molecule present in NANBH infections that consists of at least 10,000 nucleotides and that is positive-stranded with respect to the encoded NANBH antigen. These data indicate that this clone is derived from the genome of the NANBH agent and are consistent with the agent being similar to the togaviridae or flaviviridae. This molecular approach should be of great value in the isolation and characterization of other unidentified infectious agents.

WITH THE DEVELOPMENT OF SPECIFIC diagnostics for the hepatitis A virus (HAV) and the hepatitis B virus (HBV) in the 1970s, it became clear that most cases of hepatitis arising from blood transfusion were not caused by infections with these or other known viral agents (*1–4*). Despite over a decade of research, the agent or agents responsible for this so-called non-A, non-B hepatitis (NANBH) remains unidentified (*5, 6*), although there is evidence that one blood-borne NANBH agent may be a small, enveloped virus that is readily transmissible to chimpanzees (*7, 8*). A major impediment to progress in studies of this virus has been that despite intensive work, conventional immunological methods have consistently failed to identify specific viral antibodies and antigens (*5, 6*). Although this failure could be interpreted in terms of a lack of viral antibody, we consid-

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cred it to be due more likely to insufficient concentrations of viral antigen in NANBH infections.

Therefore, in order to increase viral antigen concentrations, a cDNA library derived from infectious material was constructed in the bacteriophage λgt11. This vector allows the efficient expression of cDNA-encoded polypeptides and was designed originally to facilitate the isolation of cDNA clones by means of well-characterized antibodies that bind to clones synthesizing the polypeptide of interest (*9*). This library was then screened for rare clones expressing viral antigen with serum from a chronic NANBH patient as a presumed source of viral antibodies. To increase the probability of detecting viral clones, the cDNA library was derived from chimpanzee plasma containing a relatively high infectious titer (*10*). This plasma was subjected to extensive ultracentrifugation in order to ensure the pelleting of a small virus, and nucleic acid was recovered from the pellet. Since the nature of the genome was unknown, the recovered nucleic acid was completely denatured before synthesizing cDNA from both RNA and DNA with random primers of reverse transcriptase. Screening $\sim 10^6$ of the resulting recombinant λgt11 phage led to the identification of positive cDNA clone 5-1-1.

To investigate its potential viral origin, a larger overlapping clone (clone 81) was first isolated from the same library, and the cDNA was hybridized to human and chimpanzee DNA by Southern blot analyses. This cDNA did not hybridize either to control human DNA or to DNA derived from two chimpanzees with NANBH infections (Fig. 1A). In a control experiment, the single-copy interferon gene from human fibroblasts yielded clear hybridization signals under identical conditions (Fig. 1B). This proves that clones 5-1-1 and 81 are not derived from the host genome and that DNA replication intermediates related to these sequences were undetectable.

Whereas homologous DNA sequences were undetectable, total RNA extracted from infectious chimpanzee liver hybridized specifically to the cloned cDNA but not total RNA derived from control, uninfected chimpanzee livers (Fig. 2A). The abundance of homologous RNA in total liver RNA from the infected animal was estimated to be ~0.00001% (w/w). Furthermore, total nucleic acid extracted from ultracentrifuged pellets of the high-titer NANBH chimpanzee plasma hybridized to these clones, but this hybridization signal was lost after treatment with ribonuclease but not deoxyribonuclease (Fig. 2B). Hence, it appears that these clones are derived from an exogenous RNA molecule associated with NANBH infection. This RNA from infectious plasma appears to be single-stranded since only one of the strands in clone 81 cDNA could hybridize to it even though both strands hybridized with equal efficiency to the double-stranded clone (Fig. 2C). To analyze the size of the RNA homologous to these cDNA clones, we separated RNA derived from infectious chimpanzee liver by electrophoresis through a denaturing formaldehyde agarose gel, transferred it to nitrocellulose, and hybridized it with clone 81 cDNA. Although the hybridization signal obtained with total RNA was weak, there was strong hybridization to a heterogeneous population of RNA molecules that bound to oligo(dT)-cellulose (Fig. 2D). The approximate size of this RNA was estimated to be between 5,000 to 10,000 nucleotides. T observed smear may reflect degradation d ing preparation and the maximum size the original RNA may be at least nucleotides. The binding to oligo(dT) ... lose indicates that there is either a 3′ terr nal polyadenylate sequence or an A-r tract elsewhere in the molecule.

The nucleotide sequence of clone 5-1 indicated that the cDNA strand encodi the immunoreactive polypeptide possess one continuous, translational open readi frame (ORF) (the sequence of the genor is being completed and will be deposited the GenBank database shortly). To inves gate the relation of this polypeptide w NANBH, this ORF was expressed in bac ria as a fusion polypeptide with hum superoxide dismutase (SOD) and immun blot analyses were performed on total b terial lysates. The chronic NANBH patie serum used originally to detect clo l reacts specifically with this SOD/5-1-1 f

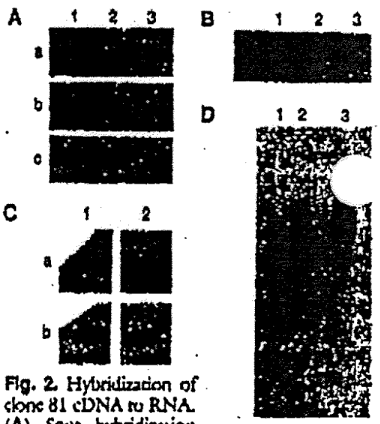

Fig. 2. Hybridization of clone 81 cDNA to RNA. (A) Spot hybridization (*24*) of 2, 4, or 12 μg of total liver RNA ... ct (*25*) from either chronic NANBH-infect... lir 910 (a1 to a3) or from two control, uninfect animals (b1 to b3 and c1 to c3) with ^{32}P-label nick-translated clone 81 cDNA. (B) Spot hybridiz tion of nucleic acid extracted from viral plasr pellets (22) before (spot 1) or after treatment wi either excess deoxyribonuclease I (spot 2) or rib nuclease A (spot 3). Hybridization probe as in (A (C) Each strand of clone 81 cDNA was subclon into phage M13mp18 and then labeled by incuba ing with Klenow *Escherichia coli* DNA polymerase in the presence of hybridization probe primer (N England Biolabs) and [α-^{32}P]dCTP (*23*). Ea probe was then hybridized to slot blots containi either identical portions of viral RNA derived fro infectious plasma (a1 and b1) or 2 pg of purifi clone 81 double-stranded cDNA (a2 and b2). (I Northern blot analysis (*26*) of 30 μg of total RN (track 1), 30 μg of unbound RNA (track 2), and 2 μg of bound RNA (track 3) after chromatograph on oligo(dT)-cellulose (Collaborative Re RNA was derived from the liver of in chimpanzee 910. Arrows indicate the relative m gration of 28S and 18S ribosomal RNA. ^{32} labeled nick-translated clone 81 cDNA was used the hybridization probe.

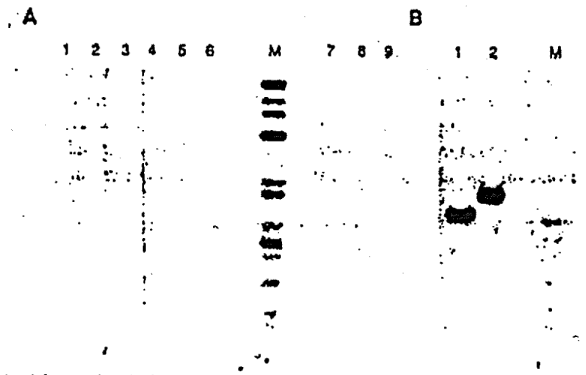

Fig. 1. Hybridization analysis of clone 81 cDNA with host DNA. (A) Southern blot containing 10 μg of DNA extracted either from a human placenta (lanes 7 to 9) or from proven infectious liver samples obtained from chimpanzees 1002 (lanes 1 to 3) and 910 (lanes 4 to 6) during acute or chronic infection, respectively, with the NANBH agent contaminating a human factor VIII concentrate (*10, 17*). Each DNA was digested with either Hinc II, Mbo I, or Eco RI before Southern blot analysis (*19*) with ^{32}P-labeled nick-translated clone 81 cDNA as the hybridization probe (*20*). M represents DNA standards of between 0.1 and 23.1 kbp. (B) Control blot of 10 μg of human placental DNA restricted with either Mbo I (lane 1) or Eco RI (lane 2) and hybridized with ^{32}P-labeled human β1-interferon cDNA (0.6 kbp) (*21*). Clone 81 was derived (*22, 23*) from an infectious plasma pool ($\geq 10^6$ chimp infectious doses per milliliter). This pool was prepared from chimpanzee 910 during chronic infection, which resulted from inoculation of chronic-phase plasma from chimpanzee 771. The latter animal was inoculated with a human factor VIII concentrate previously implicated in NANBH transmission (*10, 18*).

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sion polypeptide (PS5), whereas there was no reaction with control lysates expressing SOD alone (Fig. 3A). Similar results were obtained with serum from 7 other NANBH patients of 11 tested, whereas serum from 10 normal donors were all negative (*11*). In addition, four chimpanzees experimentally infected with the NANBH agent all seroconverted to PS5 antibody after acute infection, whereas seven animals infected with either HAV or HBV showed no such seroconversion (see Fig. 3B for representative examples). Sera from these animals were also assayed with a radioimmunoassay containing purified PS5 to capture and measure reactive antibodies. Only those animals experimentally infected with the NANBH agent developed PS5 antibodies (Table 1). These data along with results from a large study of well-characterized patients (*12*) demonstrate that the polypeptide encoded by the clone 5-1-1 ORF is closely associated with NANBH infections. Furthermore, the DNA strand that hybridized with plasma-derived RNA (Fig. 2C) was complementary to the strand encoding this 5-1-1 ORF, indicating therefore that this RNA is positive-stranded with respect to translation of this apparent viral antigen.

Thus, our data indicate that clones 5-1-1 and 81 are derived from the genome of a blood-borne NANBH virus that we now term the hepatitis C virus (HCV). Previous filtration studies have indicated that this virus is less than 80 nm in diameter and from its proven sensitivity to organic solvents, it would appear to possess an envelope made up of essential lipid (*7, 8*). These observations led to the suggestion that the agent may be togavirus-like (*13*). Our present data showing that the virus contains a positive-stranded RNA molecule of at least 10,000 nucleotides is consistent with it being related to the togaviridae or flaviviridae. The latter used to represent a genus in the togaviridae family but were recently elevated to their own family (*14*). The cDNA clones reported here were obtained in the absence of prior knowledge concerning the virus, the viral genome, and the presence of circulating viral antibodies. As such, this represents cloning without prior characterization of the infectious agent. This approach should be relevant to studies of other diseases in which an unknown infectious agent (viral or otherwise) might be involved.

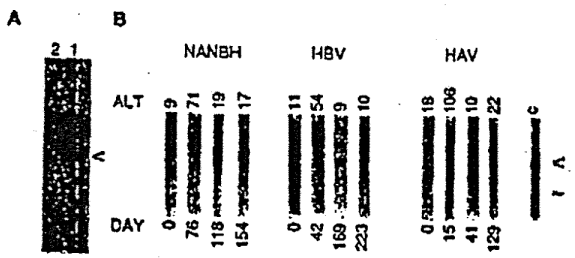

Fig. 3. Immunoblot assay for PS5 antibodies. (**A**) Incubation of the chronic NANBH patient serum used to isolate clone 5-1-1 (*22*) with blots of total bacterial lysates (*16*) containing either PS5 (lane 1) or control SOD (lane 2). (**B**) Sequential serum samples from experimentally infected chimpanzees were reacted with identical strips cut from a preparative blot of total lysate containing PS5 (*16*) to which exogenous SOD was added as an internal control. Day 0 represents the day of virus inoculation. Infections were monitored by serum ALT concentrations (international units per liter). Strip C was incubated with the same patient serum used in (A). The arrow and bar indicate the positions to which PS5 and SOD migrate, respectively.

Table 1. Incidence of PS5 antibodies in experimentally infected chimpanzees. Radioimmunoassays were performed as described (*15, 16*) on four serial serum samples obtained from each animal beginning with a sample obtained immediately prior to intravenous administration of virus (day 0). The mean of quadruplicate assays at each time point is shown (counts per minute). Values above 990 cpm (mean of uninfected controls plus three standard deviations) are considered positive. Animals 1, 7, and 8 were the same as used in Fig. 3. Animals 1 to 4 represent the third, second, fifth, and third chimpanzee passages, respectively, of the human factor VIII–derived NANBH agent (*17, 18*). The serum alanine aminotransferase (ALT) levels on the four sampling days are shown. Sampling times are represented as the number of days after inoculation of virus. nd, not done.

Chimp	Agent	Sampling times	ALT	Counts per minute
1	NANBH	0, 76, 118, 154	9, 71, 19, 17	250, 306, 5664, 8301
2	NANBH	0, 21, 73, 138	5, 52, 13, 13	294, 398, 2133, 8632
3	NANBH	0, 43, 53, 159	8, 205, 14, 6	152, 349, 392, 3738
4	NANBH	0, 55, 83, 140	11, 132, 7, 7	349, 267, 392, 2397
5	HBV	0, 359, 450	12, nd, 6	804, 660, 656
6	HBV	0, 115, 205, 240	9, 126, 9, 13	618, 606, 514, 790
7	HBV	0, 42, 169, 223	11, 54, 9, 10	454, 221, 272, 198
8	HAV	0, 15, 41, 129	18, 106, 10, 22	256, 597, 266, 295
9	HAV	0, 22, 115, 139	7, 83, 5, 10	218, 176, 214, 341
10	HAV	0, 26, 74, 205	15, 130, 8, 5	162, 219, 554, 284
11	HAV	0, 25, 40, 268	4, 147, 18, 5	333, 453, 419, 358

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16. Clone 5-1-1 cDNA was subcloned into bacterial plasmid pSODcf1 [K. S. Steimer *et al.*, *J. Virol.* 58, 9 (1986)] in order to synthesize PS5 in which the COOH-terminus represents the polypeptide encoded by the clone 5-1-1 ORF. Subclones in the opposite orientation served as control in which SOD was synthesized but not the 5-1-1 ORF–encoded polypeptide. Immunoblots of total bacterial lysates were performed [A. J. Weiner *et al.*, *J. Virol.* 62, 594 (1988)] with ^{125}I-labeled sheep antibody to human immunoglobulin (Amersham) to detect bound antibody.
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22. Plasma was first diluted 1:5 in 50 m*M* tris-HCl, pH 8.0, 1 m*M* EDTA, 100 m*M* NaCl before clearing (10,000*g* for 20 min, at 20°C) and then ultracentrifuging the supernatant (104,000*g* for 5 hours at 20°C in a Beckmann SW28 rotor). Nucleic acid was extracted [D. W. Rowe *et al.*, *Biochemistry* 17, 1581 (1978)] from the crude viral pellet and denatured with 10 m*M* CH_3HgOH prior to synthesizing cDNA (from both DNA and RNA) with random primers of reverse transcriptase (*23*). After cloning into λgt11, the resulting cDNA library was immunoscreened [T. V. Huynh, R. A. Young, R. W. Davis, in *DNA Cloning: A Practical Approach*, D. Glover Ed. (IRL Press, Oxford, UK, 1985), vol. I, pp. 49–78] with a 10^{-2} dilution of serum from a patient that had elevated serum ALT levels for more than 6 months (up to 1000 international units per liter) in the absence of serologic markers of HAV and HBV infection [immunoglobulin M (IgM) antibody to HAV, hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen, and antibody to HBsAg]. Positive plaque 5-1-1 was isolated and the 155-bp cDNA insert was used (T. V. Huynh *et al.*, *ibid.*) as a hybridization probe to the same library to isolate clone 81, which contains a 353-bp cDNA consisting of the 5-1-1 cDNA plus additional flanking sequence.
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An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-B Hepatitis

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A specific assay has been developed for a blood-borne non-A, non-B hepatitis (NANBH) virus in which a polypeptide synthesized in recombinant yeast clones of the hepatitis C virus (HCV) is used to capture circulating viral antibodies. HCV antibodies were detected in six of seven human sera that were shown previously to transmit NANBH to chimpanzees. Assays of ten blood transfusions in the United States that resulted in chronic NANBH revealed that there was at least one positive blood donor in nine of these cases and that all ten recipients seroconverted during their illnesses. About 80 percent of chronic, post-transfusion NANBH (PT-NANBH) patients from Italy and Japan had circulating HCV antibody; a much lower frequency (15 percent) was observed in acute, resolving infections. In addition, 58 percent of NANBH patients from the United States with no identifiable source of parenteral exposure to the virus were also positive for HCV antibody. These data indicate that HCV is a major cause of NANBH throughout the world.

VIRAL HEPATITIS COMMONLY OCcurs in the absence of serologic markers for such known hepatotropic agents as hepatitis A virus (HAV), hepatitis B virus (HBV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV) (*1–4*). Termed non-A, non-B hepatitis (NANBH), this entity represents greater than 90% of transfusion-associated hepatitis cases in the United States, and up to 10% of transfusions have been estimated to result in NANBH (*5, 6*). More recently, the frequent occurrence of NANBH in the absence of any obvious parenteral exposure has been well documented (*7–9*). Whereas acute disease is often subclinical, at least half of NANBH infections result in chronic hepatitis, which may result in cirrhosis in approximately 20% of cases (*10*). A potential association with hepatocellular carcinoma has also been proposed (*11*). Because of the frequency and severity of NANBH, there is an urgent need to develop a direct diagnostic test for the causative agent or agents. We have recently cloned the genome of a NANBH agent (*12*), designated the hepatitis C virus (HCV), and now report the development and use of a recombinant-based assay for HCV antibodies.

Three overlapping clones were isolated by means of the cDNA in HCV clone 5-1-1, which was used as a hybridization probe to the original cDNA library (*12*). These clones have one common open reading frame (ORF) extending throughout them that encodes part of a viral antigen associated with NANBH (*12*). This continuous ORF was reconstructed from these clones and then expressed in yeast (*13*) as a fusion polypeptide with human superoxide dismutase (SOD), which facilitates the efficient expression of foreign proteins in yeast and bacteria (*13–15*). In this way, a SOD/HCV polyp tide (C100-3) containing 363 viral am acids was synthesized at high levels (~ total protein) in recombinant yeast. Af solubilization and purification, C100-3 v used to coat the wells of microtiter plates that circulating HCV antibodies in blc samples could be captured and measur Detection of bound antibody was achiev with a radioactive second antibody.

Initially, to test the specificity and sen tivity of this assay, sera of known NANI infectivity was assayed in a blind fashi (Table 1). This panel of well-pedigreed a well-characterized samples has been acce ed widely as a crucial test of the validity putative specific assays for NANBH (*1* Of seven NANBH serum samples shown be infectious in chimpanzees, all but o gave very high signals in the assay as co pared to the results obtained with sera fr two control patients with alcoholic hepati or primary biliary cirrhosis and five nc infectious normal blood donors. These sults were reproducible in quadruplic analysis (Table 1). The only proven inf tious sample that was negative in the as was obtained from an individual in the ac phase of post-transfusion NANBH (P NANBH), although another acute-phase rum of unproven infectivity was simila negative. A blood donor implicated in tra mission of NANBH but whose serum w of equivocal infectivity in chimpanzees w also found negative in this assay. Thus, t data from this panel of sera indicates a hi sensitivity and specificity of the antibo assay for blood-borne NANBH. No oth assay evaluated by this panel has achiev this degree of specificity and sensitivity (*1*

Next, we assayed matched blood don and prospectively obtained recipient se from ten well-characterized cases of chro PT-NANBH in the United States. The r sults of the HCV antibody assays of sequ tial samples taken at 3-month intervals fr each recipient during the development NANBH and in stored samples from t corresponding donors are shown (Table Each of the ten recipients seroconvert against HCV during the course of disea although seroconversion in case 4 was m ginal and not apparent until 12 months af transfusion. In contrast, seroconversi against HCV was not observed in prospe tively studied individuals infected with oth viral hepatitis agents. Antibody seroconv sion was generally detectable within months of transfusion. The prolonged int val to antibody development may expla the observed absence of HCV antibodies the acute-phase samples assayed in Table

With one exception, significant levels HCV antibody were detected in at least o

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Table 1. Detection of HCV antibodies in proven infectious blood samples. Assays were performed (22) under code and in quadruplicate on a panel (16) containing sera from three patients with biopsy-proven chronic PT-NANBH, three implicated blood donors, and one patient with acute PT-NANBH, all of which had been proven to transmit NANBH to chimpanzees. Also included were sera from a patient with acute NANBH and a donor thrice implicated in NANBH, each of which were equivocally infectious in the chimpanzee. Control sera were assayed from five normal blood donors who had each donated blood on at least ten occasions without the development of NANBH in the recipients, from a patient with alcoholic hepatitis, and from an individual with primary biliary cirrhosis. Sera scoring positive in these assays were negative when purified SOD was used to coat wells instead of C100-3. Such samples were also positive in immunoblot analyses containing recombinant HCV polypeptides, but not SOD alone (12).

Serum	Counts per minute			
Proven infectious in chimp				
Chronic NANBH patients				
1 (PT-NANBH)	31,962	32,107	32,121	28,584
2 (PT-NANBH)	22,871	17,483	21,623	19,863
3 (PT-NANBH)	25,381	20,983	21,039	20,047
Acute PT-NANBH patient	909	726	767	580
Implicated blood donors				
1	40,883	33,521	35,870	34,526
2	25,812	23,512	26,476	23,723
3	31,495	30,907	33,723	33,043
Unproven infectivity in chimp				
Acute PT-NANBH patient	1,207	740	1,786	1,489
Implicated blood donor	590	469	477	461
Pedigreed normal controls				
Blood donors				
1	998	775	647	584
2	887	632	561	469
3	591	446	459	327
4	634	533	758	649
5	584	531	553	429
Disease controls				
Alcoholic hepatitis	842	571	586	566
Primary biliary cirrhosis	915	1,118	741	750

Table 2. Detection of HCV antibodies in the blood donors and recipients of ten cases of chronic PT-NANBH from the United States. There were 138 blood donations of apparent negativity that closely followed a normal distribution with a mean of 1536 cpm (range, 187 to 3097 cpm) and a standard deviation (SD) of 671 cpm. Samples >3549 cpm (mean + 3 SD) are considered positive. All prospectively studied blood recipients developed chronic NANBH as diagnosed by the persistent elevation of serum ALT levels (>6 months) in the absence of immunoglobulin M antibody to HAV, HBV surface antigen (HBsAg), antibody to HBsAg and HBcAg, and serologic markers for CMV and EBV infection. Biopsies from all ten patients confirmed the diagnosis of chronicity. Recipient sera were assayed at 3-month intervals (0 represents a sample obtained immediately before transfusion). Control samples consisted of sera from a prospective study of male homosexuals (23) that were assayed for up to 1 year after the onset of hepatitis as a result of infection with either HAV (18 cases), HBV (20 cases), or CMV (5 cases). None of these disease controls showed positive seroconversion to anti-HCV. The results of every positive donor unit are shown.

Case	Number of donors per transfusion	Anti-HCV assay (cpm)				
		Positive donors	Recipients (months)			
			0	3	6	12
1	18	3,910	1,870	3,220	13,120	26,780
2	18	4,590	2,530	1,170	11,400	20,750
		3,800				
3	13	6,140	1,800	1,850	14,990	4,720
4	18	None	1,430	1,370	750	4,260
5	16	24,420	2,230	790	13,960	22,020
6	11	6,080	2,100	10,160*	21,490	24,900
		25,600				
7	15	15,970	2,120	2,090	10,470	16,140
8	20	13,240	1,920	2,860	8,160	22,510
9	8†	32,790	3,370	5,800*	4,700	11,380
10	15	20,430	1,530	5,830*	19,960	20,580
		19,760				

*These moderately high counts per minute were shown in additional studies to be due to passive transfer of antibody from donors who had high antibody titers. †Only six of the eight donors were assayed.

donor to each of the ten recipients with NANBH (Table 2). Case number 4 had no positive donors and represented the recipient with the weakest seroconversion observed. Some of the positive donors had surrogate markers for NANBH [elevated serum alanine aminotransferase (ALT) concentrations or the presence of antibody to the hepatitis B core antigen (HBcAg), or both (6, 17–19)]. The prevalence of HCV antibody in voluntary blood donors from New York with normal ALT levels (<45 international units per liter) and no antibody to HBcAg was about 0.5% (2 of 412). This frequency increased to 44% (16 of 36) in donors with both elevated ALT levels and antibody to HBcAg (20).

These data from characterized NANBH panels combined with previous data (12) indicate a specific association between HCV antibody and blood-borne NANBH. This conclusion was also supported from assays of other chronic PT-NANBH patients (Table 3). These cases differ from the NANBH cases cited in Tables 1 and 2 in that they were not prospectively monitored from the time of transfusion and, in many cases, only one serum sample was assayed. This may account for the observed lower prevalence of HCV antibody.

Table 3. HCV antibody in NANBH patients from the United States.

Transmission	Total patients	Percent positive
Blood transfusion	24	71*
No identifiable source (community-acquired)	59	58†

*Between one and three serum samples assayed from patients who had received transfusions and who were diagnosed with chronic NANBH on the basis of clinical symptoms, elevations of serum ALT for >6 months, serologic exclusion of infection with other agents (Table 2), and the exclusion of other apparent causes of injury. †Sequential serum samples obtained prospectively up to 3 years after the onset of clinical hepatitis associated with elevated serum ALT in the absence of serologic markers for other agents (Table 2) and other identifiable causes of liver injury.

Table 4. HCV antibody in PT-NANBH cases from Italy and Japan.

Country	Number of patients	Disease	Percent positive
Italy	32	Chronic	84*
Japan	23	Chronic	78†
Japan	13	Acute, resolving	15†

*Serum samples (about three) assayed from each patient with transfusion-related chronic NANBH (diagnosed as in Tables 2 and 3). †A prospective study in which sequential serum samples were assayed for at least 6 months after the onset of acute NANBH (diagnosed in Tables 2 and 3). The serum ALT of acute, resolving patients returned to normal and stable levels, whereas chronic patients displayed abnormal levels for at least 6 months.

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Assays were also performed on a group of patients with well-defined clinical NANBH who were prospectively monitored for up to 3 years after onset of illness but who had no identifiable source of infection (*9*). More than 50% of these individuals were either positive for HCV antibody at the time of the initial consultation with the physician or seroconverted subsequently (Table 3). Thus, it appears that HCV is a major cause of community-acquired NANBH as well as PT-NANBH.

To initiate investigations into the contribution of HCV to global NANBH, a collection of sera from NANBH patients from Italy and Japan was assayed for HCV antibody. The results indicate that 84% of Italian patients diagnosed with chronic PT-NANBH contained HCV antibody (Table 4). A similar frequency was observed in prospectively studied chronic PT-NANBH cases from Japan, but a much lower prevalence was seen in Japanese patients with NANBH that had resolved their acute infection without progression to chronic hepatitis (Table 4). The lower incidence of antibody to HCV in acute, resolving NANBH has also been observed in other human studies (*21*) and may reflect a lower stimulation of the immune system in these cases as compared with chronic, persistent infections.

These data suggest that HCV is a major cause of chronic NANBH throughout the world. The advent of the specific, sensitive test for HCV antibody described here should improve the safety of the world's blood supply as well as provide an important clinical diagnostic tool. With this assay and the availability of HCV hybridization probes (*12*), it should also be possible to address the issue of whether other parenteral NANBH agents exist.

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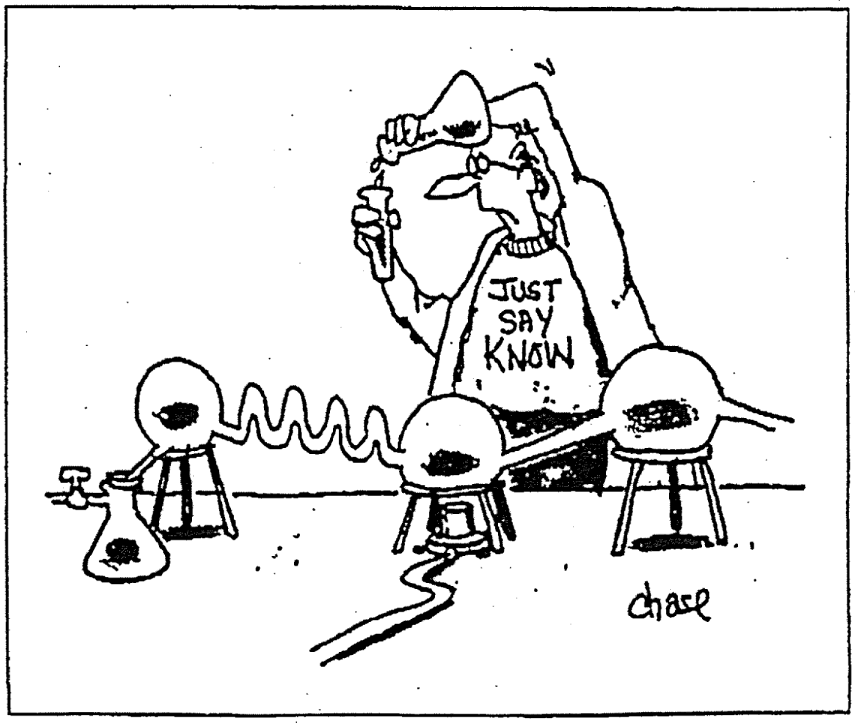

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