Clinical Medical Research Award

Hepatitis C Virus and eliminating post-transfusion hepatitis

HARVEY J. ALTER & MICHAEL HOUGHTON

You'll wonder where the yellow went: A 30-year perspective on the near-eradication of post-transfusion hepatitis

HARVEY J. ALTER

The story I will relate here traces the near-total eradication of transfusion-associated hepatitis over the course of three decades. I am perhaps the thread that links these events, but the story is a fabric woven by many collaborators who played essential parts and by the conducive environment of the National Institutes of Health (NIH) intramural program that has nurtured these clinical investigations. The story and my research career began in the early 1960s when, as an NIH Clinical Associate, I investigated the non-cellular causes of febrile transfusion reactions by screening multiply transfused patients for antibodies against serum proteins using agar gel diffusion. Ouchterlony plates were piled high on my lab bench, the way unread manuscripts are today. One day Richard Aster told me that he had heard an interesting lecture by Baruch Blumberg, who was using similar methodology to investigate protein polymorphisms. I visited Blumberg and began a collaboration that within a year uncovered an unusual precipitin line resulting from the reaction between sera from a patient with hemophilia and an Australian Aboriginal person (Fig.1). The line was unusual in that it stained only faintly with lipid dyes, in contrast to the lipoprotein polymorphisms that were then being studied. Because it stained red with the azocarmine counter-stain, we initially called this the red antigen, then debated calling it the Bethesda antigen and ultimately called it the Australia antigen, based on the evolving nomenclature for new hemoglobin discoveries. Subsequent investigations showed the prevalence of Australia antigen to be only 0.1% in the donor population, but very high (10%) in patients with leukemia. The first publication on the Australia antigen[1] cited this association with leukemia in the title. We considered that the antigen might be a component of the long-postulated leukemia virus. In retrospect, the antigen merely reflected the high transfusion exposure and the immunocompromised status of these patients. My initial first-author publication was the biophysical characterization of the Australia antigen[2].

In 1964, I left the NIH to complete my training in internal medicine and hematology, and Blumberg moved to the Institute for Cancer Research in Philadelphia, where he continued to pursue the importance of the Australia antigen. Both serendipity and good science led, by 1968, to the linkage of this antigen to viral hepatitis, a link that transformed the study of hepatitis, protected the blood supply, led to a hepatitis B vaccine and culminated in the Nobel prize.

I returned to the NIH in 1969 to investigate the causes and prevention of post-transfusion hepatitis and to pursue clinical investigations of hepatitis B and its associated antigens. I had no premonition that this would be a lifetime endeavor. My first function was to continue and expand on prospective studies of post-transfusion hepatitis initiated by John Walsh, Bob Purcell, Paul Holland and Paul Schmidt. Walsh and colleagues had already demonstrated the inordinately high hepatitis risk of blood transfusion and, particularly, the risk of paid-donor blood. In 1970, Holland, Schmidt and I, in a still-memorable meeting that would later influence national blood policy, decided that the continued use of paid donors could not be tolerated and also concluded that we should introduce donor screening for what was by then called the hepatitis-associated antigen. I then simultaneously did a retrospective analysis that demonstrated the value of hepatitis B antigen testing and initiated a new prospective study to assess the effect of this dual change in the donor supply. The result was substantial: hepatitis incidence among patients undergoing open-heart surgery plummeted from 33% to 9.7% (ref. 3) (Fig. 2). We calculated that the main determinant of this reduction of about 70% was the exclusion of paid donors. Indeed, retrospective testing for hepatitis B virus (HBV) markers showed that only 20% of the hepatitis found before antigen screening was related to HBV. The recognition of transfusion-associated non-B hepatitis therefore evolved. Improved hepatitis B antigen assays brought hepatitis B transmission to near zero by 1977. In 1973, Steve Feinstone, who had the unenviable task of sifting through stool specimens in the bowels of NIH building 7, used immune electron microscopy to discover the hepatitis A virus, in collaboration with Al Kapikian and Bob Purcell[4]. We immediately delved into our repository of non-B hepatitis cases and were surprised to find that not a single case was due to hepatitis A virus[5]. In a less-than-brilliant foray into nomenclature, we designated these cases non-A, non-B (NANB) hepatitis. Bob Purcell, in particular, felt that we should not call the agent the hepatitis C virus until we had proved transmissibility and until we established the number of agents that might be involved. In our optimism, we did not suspect that the designation non-A, non-B would persist for 15 years before its specific etiology could be defined.

Fig.1 An Australian Aborigine (left) and the precipitin line formed between the aboriginal serum and that of a multiply transfused patient with hemophilia (right). The precipitin failed to stain for lipid, but stained red with the azocarmine counter-stain for protein.

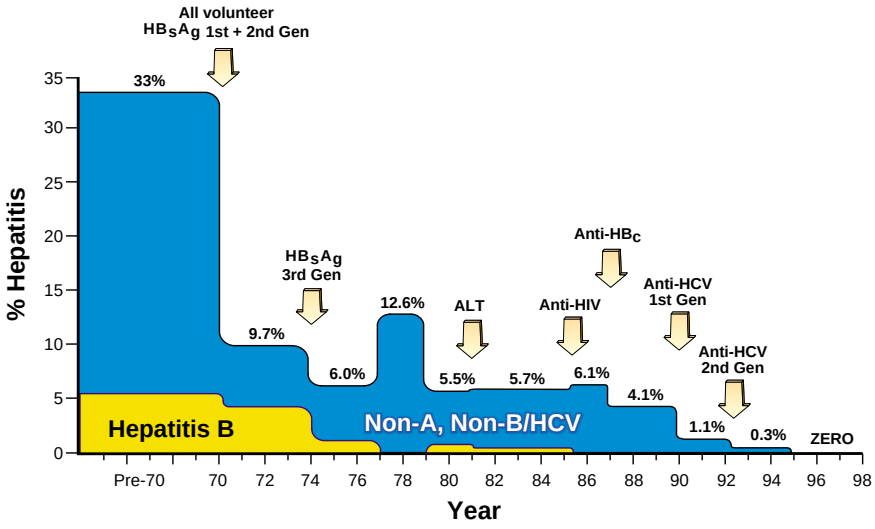

Fig. 2 The decreasing incidence of transfusion-associated hepatitis in blood recipients monitored prospectively. Incidence, traced from 1969 to 1998, demonstrates a decrease in risk from 33% to nearly zero. Arrows, main interventions in donor screening and selection that effected this change.

In 1975, as the prospective studies continued, my attention was directed at proving NANB hepatitis transmission in the chimpanzee model. Earlier attempts at chimpanzee transmission had failed, but I reasoned that we could use the highly pedigreed samples from our prospective studies and use inocula in volumes equivalent to blood transfusion in humans. We achieved success in our first attempt, as five of five chimpanzees developed increases in alanine aminotransferase (ALT) at appropriate intervals after inoculation[6]. We were later able to use this same approach and accomplish the first experimental animal transmission of human immunodeficiency virus[7]. In the absence of a tissue culture system, an observed particle or a serologic assay, the availability of an animal model was essential to further study of the NANB agent. Throughout these prospective studies, my main collaborator was Bob Purcell of the National Institute of Allergy and Infectious Diseases, who provided the basic research arm necessary to propel the investigations of NANB. From among the 50 NANB hepatitis cases identified at that time, I selected a patient (H) who had a particularly severe acute NANB hepatitis and from whom we had obtained apheresis units while his ALT levels were increasing. Purcell established a titration series of the H plasma and then did infectivity studies in the chimp. H had an infectivity titer of $10^{6.5}$ CID_{50}/ml (chimp infectious $doses_{50}$), allowing us to then undertake a series of manipulations of the virus and to test their effect on infectivity. Such studies by Feinstone[8], He[9] and others showed that the NANB agent was sensitive to chloroform and less than 30 nm in diameter, based on filtration. The agent of NANB hepatitis thus seemed to be small, lipid-enveloped, blood-transmissible and responsible for most residual cases of transfusion-associated hepatitis. Subsequently, Patrizia Farci from the University of Cagliari, in collaboration with Purcell and I, did a series of chimpanzee studies[10,11] in which she mixed chronic phase serum from patient H with the acute-phase infectious inoculum and studied the infectivity of the mixture in the chimp model. These studies made the important observation that neutralizing antibodies against hepatitis C virus (HCV) develop, but are very strain-specific and, in most cases, incapable of preventing the emergence of viral variants that lead to persistent infection.

As these virologic and immunologic studies were proceeding in the late 1970s and early 1980s, my main focus was to define the clinical consequences of NANB virus infection and to establish an assay that might be amenable to blood screening. The former proved easier than the latter. The entity NANB hepatitis initially met with considerable skepticism, and some believed it caused only an irrelevant transaminitis, because few patients had recognized clinical illness. However, as we monitored patients long-term and did liver biopsies in collaboration with Jay Hoofnagle and the NIH Liver Service, it became apparent that most NANB-infected patients had biochemical evidence of chronic hepatitis, and that 20% progressed to cirrhosis over the course of one to two decades. Later, after the discovery of HCV, we expanded these natural history studies in both asymptomatic donors[12] and transfusion recipients[13], the latter in collaboration with Leonard Seeff, and confirmed that 20–30% of HCV-infected individuals have severe histologic outcomes. However, equally important, these studies showed that about 20% of HCV-infected individuals undergo spontaneous recovery and that most have an indolent, perhaps non-progressive, course. In collaboration with Farci and Purcell, we have also shown the considerable viral diversity ('quasispecies') of HCV infection and that the extent of diversity in the acute phase of illness predicted whether chronic infection would ensue[14].

The histologic studies that documented progression to cirrhosis made it even more imperative to develop a blood-screening assay. In the decade from 1978 to 1988, we attempted every permutation of serologic approaches to assay development. Despite using 'highly pedigreed' infectious specimens, 'presumed convalescent' sera, eluted fractions, purified gamma globulins and the most-sensitive radio-immune assay approaches, we were unable to develop a specific serologic test for this elusive agent. In the absence of a specific assay, we looked for 'surrogate' markers that might identify NANB carriers. The most logical approach was measuring ALT. Although a retrospective analysis of our prospective collections showed that increases in ALT in the donor correlated with hepatitis transmission[15], we were unable to show the efficacy of this 'surrogate' assay in a subsequent prospective study. We then sought other measures of donor intervention and reasoned that donors who had been exposed to HBV might also be more likely to have been exposed to NANB; such donors were likely to have recovered from HBV infection and pass the donor screen, but might be persistent carriers of NANB. Thus, we used antibody against hepatitis B core antigen (HBc) as an index of past HBV infection, and showed in a retrospective analysis of our cohort that donors with antibody against HBc were four times more likely to transmit NANB hepatitis and that their exclusion might prevent 30% of such transmissions[16]. These data and those from a multicenter collaborative transfusion-transmitted viruses study[17] convinced the main blood organizations to implement testing for antibodies against HBc and for ALT in routine donor screening in 1987. It was difficult to measure the specific effect of these 'surrogate' assays because the threat of transfusion-associated AIDS had

emerged and the 'surrogate' assays were introduced in concert with more-intensive questioning of donors regarding high-risk behavior and by a lessened use of allogeneic blood. Nonetheless, we could show that these combined measures served to decrease hepatitis incidence to 4.5% by 1989 (Fig. 2). Efforts to develop a specific NANB assay continued throughout the 1980s, although the main effort by Chiron was kept well concealed.

During this time, I had developed a panel of sera consisting of duplicate coded samples that had been proved to be infectious in the chimp or non-infectious in humans. By 1989, many different laboratories claimed to have developed a NANB assay and asked to test the panel. None was able to break the code and by 1989, the score was viruses, 20; investigators, zero. At that time, I received a call from George Kuo at Chiron, saying that they too felt they had a NANB assay. I sent George the remnants of the now-dwindling panel and within days received their results followed by several anxious calls asking if I had yet broken the code. When I did, I was excited to find that Chiron had detected all but two of the infectious sera and had properly found all the non-infectious sera to be negative. Further, the two samples that they missed were acute-phase sera, and subsequent samples from these same patients proved to be positive for what Chiron now called the hepatitis C virus. Michael Houghton will describe the events that preceded this discovery.

Using the newly developed assay for antibodies against HCV, we again delved into our repository and were able to rapidly show that 88% of NANB hepatitis cases seroconverted for antibody against HCV, that the development of antibody was in temporal relationship to the course of hepatitis and that infected patients could be linked to infected donors[18]. Thus, by 1990 it was clear that HCV was the principal agent of NANB hepatitis, and universal donor screening was initiated. We established a new prospective study to measure the effect of such testing and to define the extent of residual hepatitis unrelated to HBV or HCV. The first-generation assay for antibody against HCV resulted in a further 70% decrease in hepatitis incidence to a residual rate of 1.5%, and a more-sensitive second-generation assay, introduced in 1992, nearly eliminated HCV transmission (Fig. 2). Although mathematical modeling indicates that antibody-screened blood might still transmit HCV to 1:100,000 to 1:200,000 recipients, the observed decrease from 33% in 1970 to nearly zero in 1997 stands as a testament to the cumulative effectiveness of a series of donor screening interventions that were evidence-based. Viral nucleic acid testing of donors and improved viral inactivation technologies will soon bring transmission of hepatitis and human immunodeficiency virus from near-zero to absolute zero. I am now looking for another line of work.

Acknowledgments

Throughout almost the entire course of these clinical investigations, my right arm, and sometimes my left as well, has been my dedicated assistant, J. Melpolder. There is no way to adequately acknowledge the substantial contribution she has made in coordinating these studies that have involved thousands of patients. My gratitude is without bounds. I would also like to gratefully acknowledge the manifold contributions of my long-term associate James W. Shih, Ph.D. who so ably supervised the diverse laboratory aspects of these prospective studies.

The hepatitis C virus: A new paradigm for the identification and control of infectious disease

MICHAEL HOUGHTON

Identification of the hepatitis C virus

The problem of non-A, non-B (NANB) hepatitis emerged in 1975, after serological tests for hepatitis A virus (HAV) and hepatitis B virus (HBV) were developed. It then became evident that most hepatitis cases after transfusion were not due to either HAV or HBV, and that the risk of NANB hepatitis after blood transfusions was as high as 10% or even greater[5]. Later, it also became evident that NANB hepatitis occurred frequently in the form of sporadic, community-acquired infections. A frustrating period of 13 years followed, in which the methods successfully used to identify HAV and HBV all failed to result in the molecular identification of the etiological agent(s) of NANB hepatitis. No NANB-hepatitis-specific antigen, antibody or cell culture system was identified, and this lack of a molecular 'handle' limited progress in identifying the causative agent(s) of NANB hepatitis[19]. However, a chimpanzee model successfully developed by several groups[6,20,21] was exploited to show that one NANB hepatitis agent induced characteristic membranous tubules within the endoplasmic reticulum of infected chimpanzee hepatocytes[22]. Known as the tubule-forming agent, it was later shown to be filterable and to lose infectivity after treatment with organic solvents, consistent with its being a lipid-enveloped virus, possibly a toga-like or flavi-like virus[9,23]. Other data supported the existence of an HBV-like NANB hepatitis agent[19] as well as a chloroform-resistant (non-enveloped) NANB hepatitis virus[23] and possibly other NANB hepatitis agents[22].

Eventually, what turned out to be the main form of parenterally transmitted NANB hepatitis was identified using a 'blind' immunoscreening approach applied to recombinant λgt11 (ref. 24) cDNA libraries prepared from total RNA and DNA extracted from infectious chimpanzee plasma[25]. Serum from a patient diagnosed with NANB hepatitis was used as a presumed (but unproven) source of NANB-hepatitis-specific antibodies to identify just one viral cDNA clone from a complex cDNA library constituting one million other cDNAs. Formal proof of its etiological origin came from the demonstration that the clone was not derived from the host genome, that it bound a large RNA molecule of around 10,000 nucleotides found only in NANB-hepatitis-infectious materials, and that it was derived from a positive-stranded RNA encoding a protein that induced antibodies only in NANB hepatitis-infected individuals[25,39]. The RNA genome also encoded a large polyprotein of about 3,000 amino acids that had distant primary sequence identity with members of the Flaviviridae family[26]. HCV was therefore identified by direct molecular cloning of its genome in the relative absence of knowledge concerning the nature of the infectious agent and the immune response. This 'blind' method could be of value in the future in unearthing other unknown infectious agents involved in disease. The molecular identification of HCV was the culmination of a team effort[25] spanning 7 years, during which hundreds of millions of bacterial cDNA clones were screened for a putative NANB hepatitis origin using many different approaches. The successful approach involved Qui-Lim Choo in my Laboratory at Chiron and the laboratories of George Kuo (Chiron) and Daniel Bradley (CDC). I accept the award on behalf of these collaborators (Fig. 3).

Figure 3 The HCV team (from left to right; M. Houghton, Q-L Choo, G. Kuo and D. Bradley.

have been developed, based on the highly conserved 5′ internal ribosome entry site and nucleocapsid gene sequences, which can diagnose new infections before seroconversion to having antibodies against HCV occurs. Implementation of these tests for screening of blood donors will reduce the risk of HCV after transfusion still further (down to approximately one in 300,000 in the US, for example). A test detecting circulating nucleocapsid antigen is also of value in diagnosing infection before seroconversion.

Properties of HCV

Now known to contain a highly variable RNA genome, HCVs constitute a large genus (the hepacivirus genus) within the Flaviviridae family. Six basic genotypes have been distinguished so far, with more than 100 phylogenetically-distinct subtypes[27]. At any one time, the viral genome exists as a complex quasi-species. The RNA genome contains a conserved 5′-terminal internal ribosome entry site that is responsible for initiating translation of the large polyprotein. The latter is cleaved co- and post-translationally into at least three structural or virion proteins and seven presumed non-structural proteins involved in replication of the virus[28-30] (Fig. 4). The 3′ terminus of the RNA genome is composed of a variable region, a polypyrimidine tract and a highly conserved stem–loop secondary structure[29]. Hypervariable regions exist within the large gpE2 glycoprotein domain that may be under immune selection[31]. The virus cannot be grown efficiently in cell culture or purified from infected liver or blood, and thus still has not been characterized morphologically or biochemically.

Serodiagnosis

The molecular cloning of the HCV genome led to the availability of many recombinant HCV diagnostic antigens. George Kuo purified these and developed numerous experimental EIA tests detecting HCV antibodies. This intensive work allowed the selection of optimal, immunodominant epitopes for inclusion in an evolving series of sensitive and specific blood screening and diagnostic tests for HCV infection[32]. Used to screen blood donors beginning in 1990, these assays have led to the near-disappearance of transfusion-associated hepatitis C. At least 40,000 infections have been prevented each year in the US alone since the implementation of these tests[33]. Such tests have also been of great use in diagnosing hepatitis patients and in their clinical management, and in attributing liver and extra-hepatic diseases to HCV infection. Chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, cryoglobulinemia and porphyria cutanea tarda are all well-established potential clinical sequelae of chronic persistent HCV infection. Other diseases, such as oral lichen planus, Sjögren's-like syndrome and non-Hodgkin lymphoma, have also been linked with HCV infection[28]. Although most HCV-infected individuals have few clinical symptoms and will not progress to a severe disease state, a subset can undergo progressive liver disease in which, often over decades, chronic hepatitis develops into liver cirrhosis and then into hepatocellular carcinoma. Extra-hepatic manifestations can also occur.

Recently, various nucleic acid testing assays

Education, new therapies and vaccines

Future challenges exist for both developed and developing countries. In the latter, global implementation of blood donor screening for HCV has been recommended recently by the World Health Organization. In many countries in which HCV is endemic, the risks of HCV infection after transfusion are still exceedingly high. Also, the World Health Organization has emphasized education to lower the risks of HCV transmission in both developing and developed countries. The historical use of non-sterile injection devices has been mainly responsible for the huge burden of HCV disease present in many developing countries, as well as cultural practices (such as circumcision) involving the use of non-sterile medical equipment. In developed countries, intravenous drug use involving sharing of needles/syringes is still the main risk factor[34]. Any procedure involving blood transfer (such as tattooing using shared instruments) is not recommended. The Centers for Disease Control also recommends that HCV-infected individuals not share toothbrushes, razors and so on.

The current therapy for HCV consists of a combination of interferon and ribovirin[35]. However, both drugs can produce substantial toxicity, and only a minority of patients responds. In particular, long-term response rates with the most common genotype, type 1, occur in only approximately 30% of patients. Although the imminent introduction of a more stable form of pegylated interferon will improve response rates somewhat, it is apparent that more-effective and less-toxic drugs are required. The HCV genome encodes two proteases involved in processing of the viral polyprotein: a helicase involved in unwinding the RNA strands during replication and translation, and a replicase that copies the positive RNA strand (Fig. 4). The fine structures of all these enzymes have now been resolved by X-ray diffraction methods and are now the subjects of rational drug design[29,30].

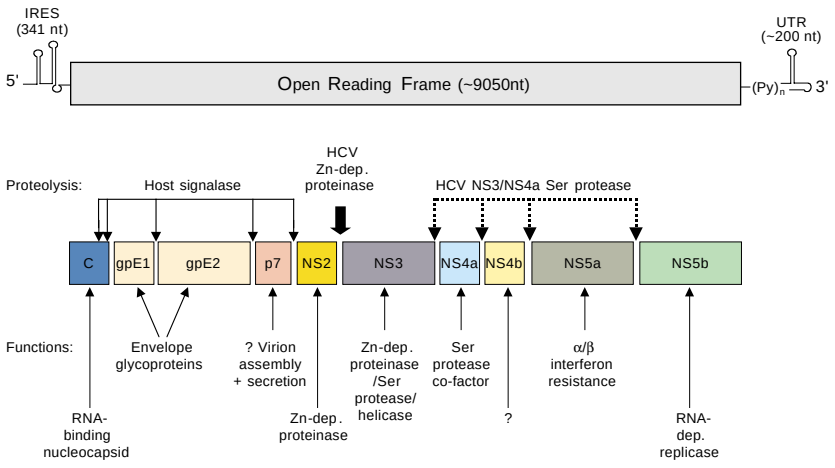

Figure 4 The organization of the hepatitis C positive-stranded RNA genome.

Other therapeutic developments involve ribozyme and antisense strategies involving the conserved 5′ internal ribosome entry site and nucleocapsid gene sequences, and nucleoside analog inhibitors of the replicase. A putative receptor for HCV, the CD81 tetraspanin molecule, is also the subject of potential antiviral development[36]. Therefore, we can be optimistic that new, specific drugs against HCV will emerge within the next 5–10 years.

Between 12 and 50% of acute HCV infections spontaneously resolve without progressing to the chronically infected state that is associated with the pathogenic sequelae of infection. Such resolution of acute infection is associated with the induction of broad helper and cytotoxic T-lymphocyte responses to the virus[37]. Thus, appropriate vaccination to prime such immune responses may lower the high chronicity rate associated with HCV infection. Vaccination of chimpanzees with recombinant envelope glycoproteins gpE1 plus gpE2 successfully prevented the development of chronic infection in most animals after challenge with homologous or heterologous subtype 1a virus. In contrast, most control unvaccinated animals develop chronic, persistent infections[37]. These data are encouraging for the development of human vaccines. Human immunoglobulin preparations (containing antibodies against HCV) have been reported to be effective at reducing the rate of development of chronic infection in the transfusion setting, between sexual partners and in liver transplantation[37]. Response to interferon has also been linked with the endogenous level of intrahepatic HCV-specific cytotoxic T-lymphocyte activity before treatment[38]. If it is confirmed, appropriate vaccination may also be important therapy if such virus-specific cytotoxic T-lymphocyte activity can be boosted during drug therapy.

Finally, although HCV is an RNA virus that does not produce DNA replication intermediates that can integrate into the host genome, it still manages to persist in most cases (usually for life in untreated individuals). Although it may involve the emergence of viral escape mutants to both antibody and T-cell responses, it is very likely that additional mechanisms are in operation to result in such high rates of chronicity. Elucidating these mechanisms represents the most intriguing challenge of future HCV research and, in the process, is likely to open up new strategies for the control of this challenging virus.

Acknowledgements

I gratefully acknowledge the input of the late Lacy Overby as well as Amy Weiner in this work.

1. Blumberg, B.S., Alter, H.J.& Visnich, S. A "new" antigen in leukemia sera. *JAMA* **191**, 541– 546 (1965).
2. Alter, H.J.& Blumberg, B.S. Further studies on a "new" human isoprecipitin system (Australia antigen). *Blood* **27**, 297–309 (1966).
3. Alter, H.J. *et al*. Posttransfusion hepatitis after exclusion of the commercial and hepatitis B antigen positive donor. *Ann. Int. Med.* **77**, 691–699 (1972).
4. Feinstone, S.M., Kapikian, A.Z. & Purcell, R.H. Hepatitis A detection by immune electron microscopy of a virus-like antigen associated with acute illness. *Science* **182**, 1026–1028 (1973).
5. Feinstone, S.M., Kapikian, A.Z., Purcell, R.H., Alter, H.J. & Holland, P.V. Transfusion-associated hepatitis not due to viral hepatitis type A or B. *N. Engl. J. Med.* **292**, 767–770 (1975).
6. Alter, H.J., Purcell, R.H., Holland, P.V. & Popper, H. Transmissible agent in "non-A, non-B" hepatitis. *Lancet* **1**, 459–463 (1978).
7. Alter, H.J. *et al.* Transmission of HTLV-III Infection from human plasma to chimpanzees: an animal model for the acquired immunodeficiency syndrome. *Science* **226**, 549–552 (1984).
8. Feinstone, J.M. *et al.* Inactivation of hepatitis b virus and non-a, non-b virus by chloroform. *Infect. Immun.* **4**, 816–821 (1983).
9. He, L-F. *et al.* Determining the size of non-A, non-B hepatitis virus by filtration. *J. Inf. Dis.* **156**, 636–640 (1987).
10. Farci, P. *et al.* Lack of protective immunity against reinfection with hepatitis C virus. *Science* **258**, 135–140 (1992).
11. Farci, P. *et al*. Prevention of HCV infection in chimpanzees following antibody-mediated in vitro neutralization. *Proc. Natl. Acad. Sci. USA* **91**, 7792 (1994).
12. Conry-Cantilena, C. *et al.* Routes of infection, viremia, and liver disease in blood donors found to have hepatitis C virus infection. *N. Engl.J. Med.* **334**, 1691–1696 (1996).
13. Seeff, L.B. *et al.* Long-term mortality after transfusion-associated non-A, non-B hepatitis. *N .Engl. J. Med.* **327**, 1906–1911 (1992).
14. Farci, P. *et al.* The outcome of acute hepatitis C predicted by the evolution of the viral quasispecies. *Science* **288**, 339–344 (2000).
15. Alter, H.J., Purcell, R.H., Holland, P.V., Alling, D.W. & Koziol, D.E. The relationship of donor transaminase (ALT) to recipient hepatitis: impact on blood transfusion services. *JAMA* **246**, 630–634 (1981).
16. Koziol, D.E. *et al*. Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Ann. Intern. Med.* **104**, 488–495 (1986).
17. Stevens, C.E. *et al*. Hepatitis B virus antibody in blood donors and the occurrence of non-A, non-B hepatitis in transfusion recipients: an analysis of the transfusion-transmitted viruses study. *Ann. Intern. Med.* **104**, 488–495 (1984).
18. Alter, H.J. *et al*. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N. Engl. J. Med.* **321**, 1494–1500 (1989).
19. Shih, J.W.-K, Esteban, J.I. & Alter, H.J. Non-A, non-B hepatitis: advances and unfulfilled expectations of the first decade. *Prog. Liver Dis.* **8**, 433–452 (1986).
20. Tabor, E. *et al*. Transmission of non-A, non-B hepatitis from man to chimpanzee. *Lancet* 1, 463–466 (1978).
21. Hollinger, F.B., Gitnick, G.L. & Aach, R.D. Non-A, non-B hepatitis transmission in chimpanzees:a project of the transfusion-transmitted viruses study group. *Intervirology* **10**, 60–68 (1978).
22. Shimizu, Y.K. *et al*. Non-A, non-B hepatitis: ultrastructural evidence for two agents in experimentally infected chimpanzees. *Science* **205**, 197–200 (1979).
23. Bradley, D.W. The agents of non-A, non-B hepatitis. *J. Virol. Meth*. **10**, 307–319 (1985).
24. Young, R.A. & Davis, R.W. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad.Sci. USA* **80**, 1194–1198 (1983).
25. Choo, Q.-L. *et al*. Isolation of a cDNA clone derived from a blood-borne non-A,non-B viral hepatitis genome. *Science* **244**, 359–362 (1989).
26. Choo, Q.L. *et al*. Genetic organisation and diversity of the hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **88**, 2451–2455 (1991).
27. Simmonds, P. Viral heterogeneity of the hepatitis C virus. *J. Hepatol*. **31**, 54–60 (1999).
28. Houghton, M. in *Fields Virology* (eds. Fields, B.N. *et al*.) 1035–1058 (Raven, Philadelphia, 1996).
29. Reed, K. & Rice, C. in *The Hepatitis C Viruses* (eds. Hagedorn, C. & Rice, C.) 55-84 (Springer, Berlin, 2000).
30. DeFrancesco, R. *et al*. Biochemical and immunologic properties of the nonstructural proteins of the hepatitis C virus: implications for development of antiviral agents and vaccines. *Semin. Liver Dis*. **20**, 69–83 (2000).
31. Weiner, A.J. *et al*. Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: potential role in chronic HCV infections. *Proc. Natl. Acad. Sci. USA* **89**, 3468–3472 (1992).
32. Kuo, G. *et al*. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* **244**, 362–364 (1989).
33. Alter, H.J. Discovery of non-A, non-B hepatitis and identification of its etiology. *Am. J. Med*. **107**, 16S–20S (1999).
34. Wasley, A. & Alter, M. Epidemiology of hepatitis C: geographical differences and temporal trends. *Semin. Liver Dis*. **20**, 1–16 (2000).
35. Davis, G.L. Current therapy for chronic hepatitis C. *Gastroenterology* **118**, S104–S114 (2000).
36. Pileri, P. *et al*. Binding of hepatitis C virus to CD81. *Science* **282**, 938–941 (1998).
37. Houghton, M. in *The Hepatitis C Viruses* (eds. Hagedorn, C. & Rice, C.) 327–339 (Springer, Berlin, 2000).
38. Nelson, D.R., Marousis, C.G., Ohno, T., Davis, G.L. & Lau, J.Y. Intrahepatic hepatitis C virus-specific cytotoxic T lymphocyte activity and response to interferon alpha therapy in chronic hepatitis C. *Hepatology* **28**, 225–230 (1998).
39. Ezzell, C. Candidate cause identified of non-A, non-B hepatitis. *Nature* **333**, 195 (1998).

Harvey J. Alter
Chief, Immunology Section, Department of Transfusion Medicine
Warren Grant Magnuson Clinical Center
NIH Building 10, Room 1C711, 9000 Rockville Pike
Bethesdsa, Maryland 20892

Michael Houghton
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608-2916