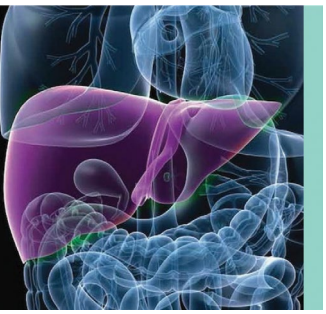

Reflections on the History of HCV: A Posthumous Examination

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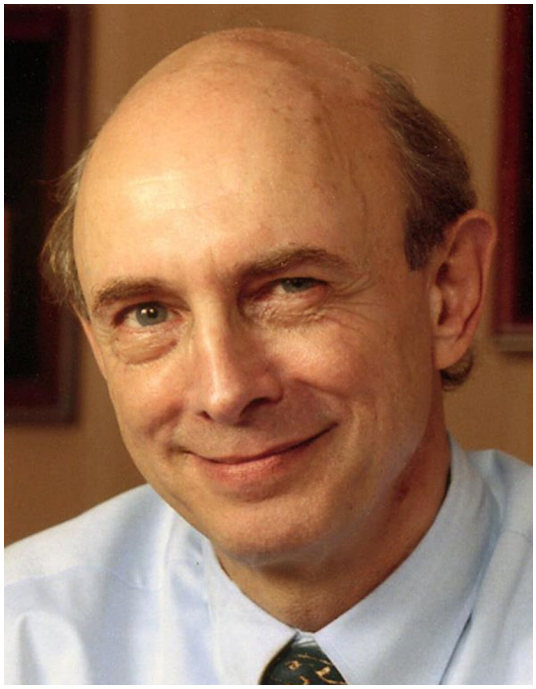

My name is Mr. H, but you can call me H. I contracted hepatitis in 1977 and died 32 years later with, but not of, the disease. I am writing this remembrance posthumously, something you will understand better when you too have "shuffled off this mortal coil," as Hamlet observed poetically when contemplating death as an exit from his troubles. My story began inauspiciously on a hike with my wife. It was our passion to blaze trails that others might follow. As it turned out, I serendipitously blazed a hepatitis trail that the whole world would follow. On this particular day in 1977, while climbing a steep hill, I developed searing chest pain and collapsed into a seeming anoxic finality. Had my wife not been with me, that would have been the last trail I blazed and the end of this abbreviated story. Perhaps going out in a blaze would not have been so bad, but my wife administered cardiopulmonary resuscitation (CPR) and revived me, following which I circuitously found my way to the cardiac surgery branch at the National Institutes of Health (NIH). There I underwent one of the pioneering triple coronary bypass surgeries under the skilled hands of the late Andrew Glenn Morrow. During and immediately after surgery, I required 17 units of blood and was enrolled in a blood bank study of posttransfusion

Abbreviations: ALT, alanine aminotransferase; CAH, chronic active hepatitis; CDC, Centers for Disease Control and Prevention; CPH, chronic persistent hepatitis; CPR, cardiopulmonary resuscitation; HCV, hepatitis C virus; HVR1, hypervariable region 1; NANBH, non-A, non-B hepatitis; NIH, National Institutes of Health.

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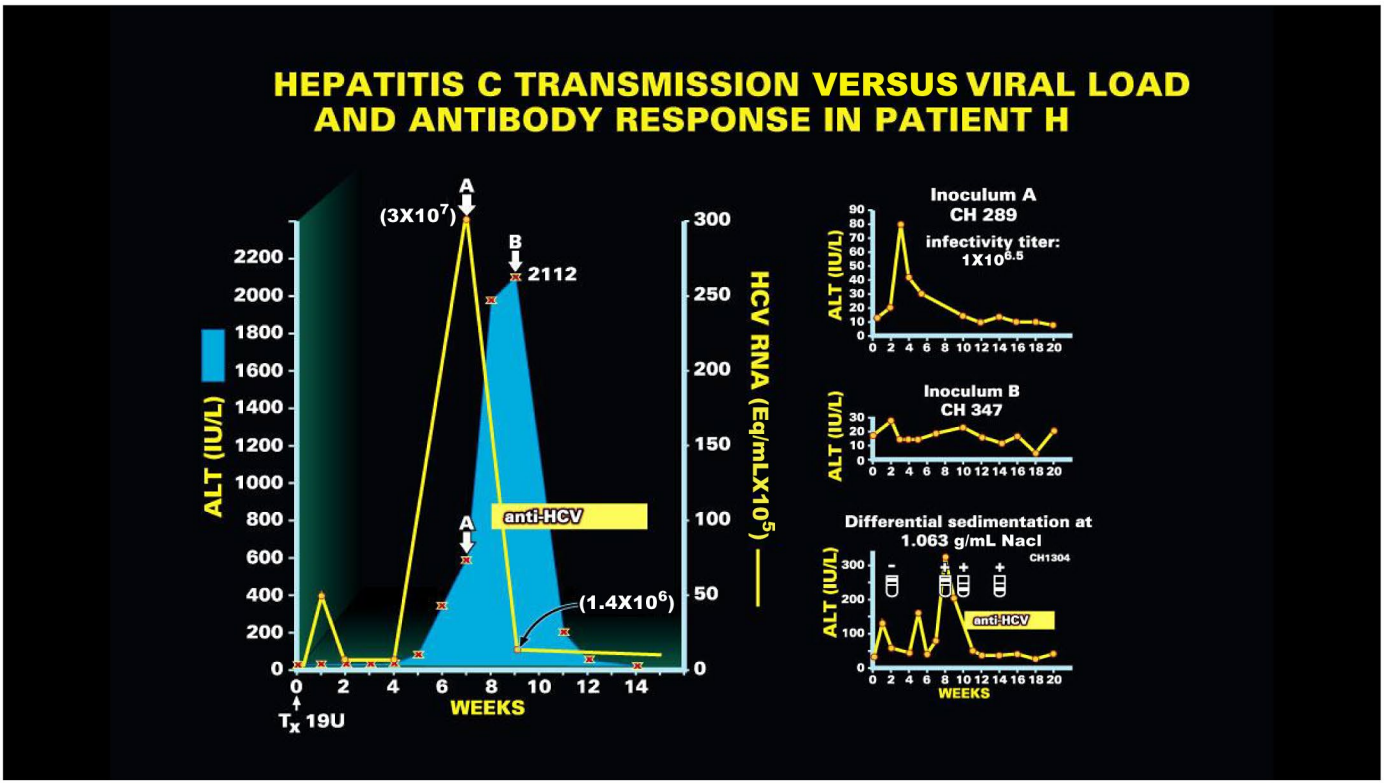

FIG 1 My early course, patient H. Six weeks after receiving 19 units of blood for open heart surgery, ALT levels (blue shading) began to rise. A plasma apheresis unit was obtained on the early rising slope of the ALT curve (point A). One milliliter of this sample was inoculated intravenously into a chimpanzee and caused hepatitis (upper right panel). Subsequently, this sample was titered in other chimpanzees and shown to have an infectivity titer of $1 \times 10^{6.5}$ chimp infectious doses per milliliter. A second apheresis unit was obtained at point B when the ALT reached a peak of 2112 IU/L. One milliliter of this sample was inoculated into a second chimpanzee but did not cause hepatitis (middle right panel), perhaps because HCV RNA was in rapid decline (yellow line) and because the virus was now immune complexed (lower right panel). Retrospective testing for HCV RNA revealed a small elevation at week 1 that was passively transferred from the infecting donor and then a rise to 3×10^7 copies/mL coincident with the peak ALT. It is clear from the graph and the subsequent course that the virus was initially contained, but not eradicated.

hepatitis. I had a rocky course and was still in the hospital 6 weeks later when a "youngish" Dr. Alter told me that my liver enzymes were rising and that I appeared to be developing posttransfusion hepatitis. This was not a particularly astute diagnosis since I was also turning yellow at the time. I was informed that I was developing non-A, non-B hepatitis (NANBH), and that since we knew very little about the causative agent, it would be very useful to obtain a large volume of my blood during the acute phase of my infection when the offending agent might be at its highest titer.

A 500-mL apheresis was performed at point A in Fig. 1; that plasma subsequently proved to be infectious in a chimpanzee (Fig. 1, top right). A second apheresis was obtained at point B, when my serum alanine aminotransferase (ALT) peaked at 2112 IU/L. Surprisingly, this sample was not infectious in a second chimpanzee (Fig. 1, middle right). In retrospect, it can be seen that at the time that my ALT peaked, my hepatitis C virus (HCV) RNA level was in a rapid decline and antibodies to HCV had become detectable. It is possible, if not probable, that the sample from

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point B was rendered less infectious because the virus was immune complexed and partially neutralized. Indeed, as shown in the bottom right of Fig. 1, sedimentation studies documented that the agent sedimented at a higher density after the appearance of antibodies. Importantly, plasma obtained during the ascending limb of my ALT curve was aliquoted into multiple vials and titered for infectivity in the chimpanzee model by Dr. Robert Purcell. My early-phase plasma was indeed uniformly infectious in the chimpanzee, and I am told had an infectivity titer of $10^{6.5}$ chimp infectious doses per milliliter, similar to retrospective polymerase chain reaction testing that showed a viral titer of 3×10^7 copies/mL. I am proud that this material was designated the "H strain" of NANBH and was distributed to numerous laboratories throughout the world. Although others received credit for this work, you know now that it was I who made it happen. In the place where I now reside, I get all the credit.

I am also proud that my infectious plasma when coupled to the chimpanzee model allowed experiments that were highly informative. Steve Feinstone and colleagues[1] extracted my plasma with chloroform and showed that this prevented hepatitis in the chimpanzee, whereas a mock extracted control sample was highly infectious (Fig. 2). Hence it could be inferred that the NANBH agent was lipid enveloped and, in retrospect, that solvent detergent could be used to inactivate infectious plasma. Le Fang He et al.[2] in the Purcell laboratory then performed filtration studies to show that the NANBH agent was between 30 and 60 nm in diameter. Hence even before definitive cloning that was to occur a decade later, it was most likely that the NANBH agent was either a small RNA virus or an entirely new class of infectious agents. I am told that Daniel Bradley at the Centers for Disease Control and Prevention (CDC), who was performing parallel studies of the NANBH agent in chimpanzees, was the first to propose that the NANBH virus was most likely a flavivirus, as it proved to be.

After the acute phase of my illness, I had no symptoms related to my hepatitis, and my ALT level fluctuated between normal and 1.5 times the upper limit of normal. Nonetheless, it was suggested that I undergo liver biopsy,

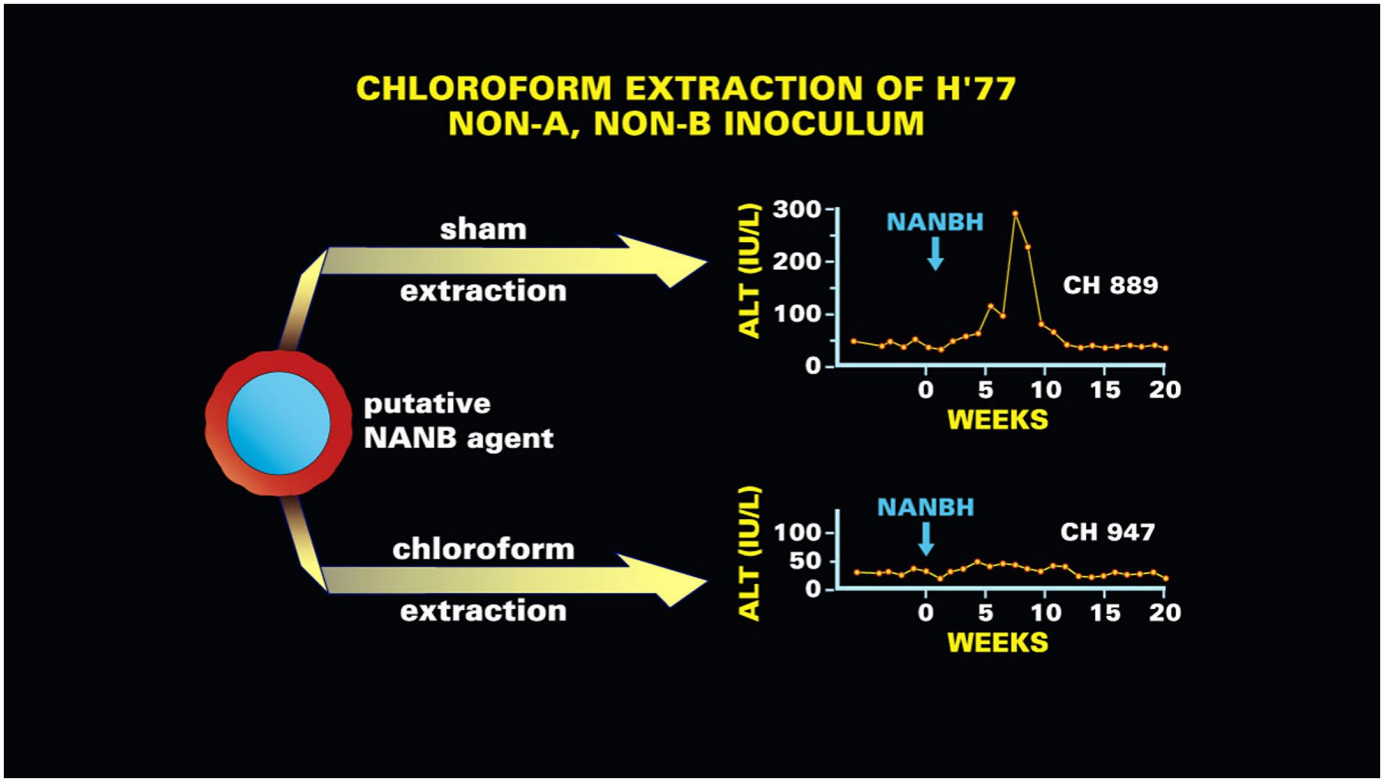

FIG 2 Chloroform extraction study. The H77 infectious inoculum was extracted with chloroform and then inoculated intravenously into a chimpanzee. The chloroform-treated inoculum did not cause hepatitis, whereas a sham extracted inoculum caused unequivocal hepatitis in a second chimpanzee as indicated by both ALT elevation and liver biopsy (upper right panel). Adapted from *Infection and Immunity*.[1]

to which I consented because I was fully committed to this path of discovery. It was much like deciding which turn to take on an unmarked trail. My initial biopsy showed that I had mild chronic persistent hepatitis (CPH) without fibrosis. There were no pathological changes to distinguish my biopsy from any other form of viral hepatitis. However, electron microscopy studies revealed peculiar cytoplasmic tubular structures that later were shown to be characteristic of other cases of NANBH.[3] Over the course of 18 years, I underwent three more liver biopsies performed by the National Institute of Diabetes and Digestive and Kidney Diseases Liver Service, then directed by Jay Hoofnagle and his team of brilliant fellows including Adrian Di Bisceglie, Graham Cooksley, John Vierling, James Dooley, Michael Fried, and Gary Davis—a panoply of future American Association for the Study of Liver Diseases leaders. Marvin Berman and Kamal Ishak led a study that evaluated my liver biopsy and that of 38 others.[4] As shown in Fig. 3, on initial biopsy, most chronic NANBH cases had only mild-to-moderate histological activity with no or minimal fibrosis. However, 10% had cirrhosis and 13% had chronic active hepatitis (CAH) with the potential to evolve to cirrhosis. Follow-up biopsy at varying intervals in 20 of these patients showed that the majority had stable or even lessening histological lesions, but that 25% progressed to cirrhosis. In total, 8 of 39 (20%) patients developed cirrhosis, a proportion that has been relatively consistent over the decades. Importantly, three of eight patients with cirrhosis died of liver failure and three others had severe liver disease when they died of an intercurrent event (Fig. 3), thus dispelling the notion, held by some at the time, that NANBH was a mild, nonprogressive liver disorder.

Fortunately, my three subsequent biopsies over the course of nearly two decades showed no histological progression of the mild inflammation and no evolution to significant fibrosis. In retrospect, I wish I had had an autopsy to examine my liver at the time of my demise, 32 years past the onset of infection. My guess is that it would not have shown any progressive disease, based on the absence of significant ALT elevations in nearly 100

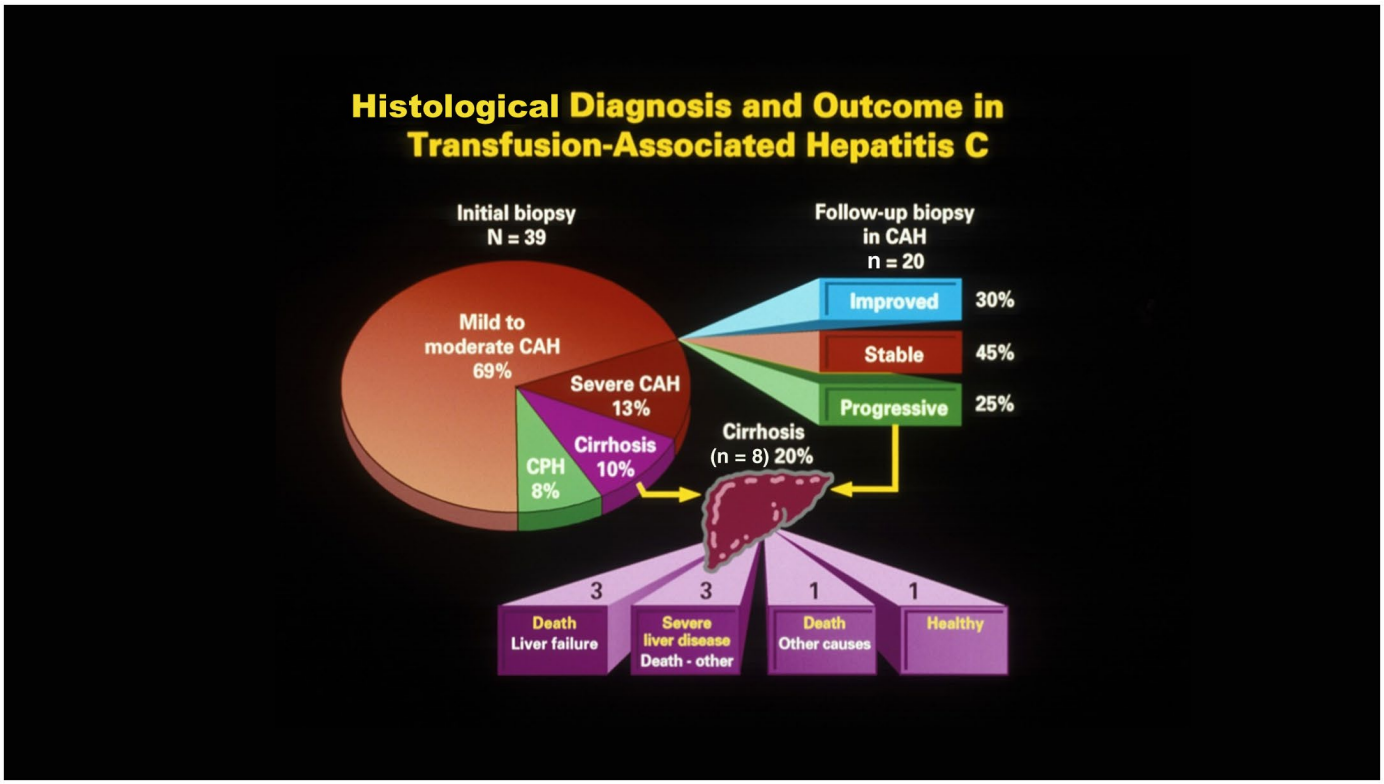

FIG 3 Histological diagnosis and outcome in transfusion-associated hepatitis C. Thirty-nine patients with NANBH/HCV initially underwent biopsy. Most had what was then termed CPH (8%) or mild-to-moderate CAH (69%). However, 10% already had cirrhosis at the time of the first biopsy and 13% had severe CAH that would likely progress to cirrhosis. Follow-up biopsy in 20 patients at varying intervals showed that 25% had progressive fibrosis and, in total, 8 of 39 patients (20%) developed NANBH/HCV-associated cirrhosis, and of these, 3 died of liver failure and 3 others had severe liver disease when they died of a cardiac or other intercurrent event.

determinations and on my general sense of well-being. Although I believe that my NANB hepatitis would have afforded me my full life span, my heart, unfortunately, died before my liver and I cannot prove my contention. Nonetheless, thanks to my wife and skilled cardiac surgeons, I lived 32 years beyond near death on a mountainside, and the HCV lived with me in a seeming symbiotic relationship in which the virus persisted in my body relatively unharmed and in which I persisted with a new and unexpected purpose in life.

Another investigator who became intimately involved with my virus was Dr. Patrizia Farci, who demonstrated the extent and nature of the viral quasispecies that I harbored and the role of immune pressure on the evolution of viral variants. Previously, Ogata et al.[5] in the Purcell laboratory had sequenced my viral genome and identified hypervariable region 1 (HVR1) of the E2 envelope protein as the most variable and mutation-prone region of the genome. Dr. Farci, in characterizing 104 clones from the HVR1 region of my 1977 acute-phase sample (Fig. 4), showed that 67% of sequences represented a dominant clone, but that 18 other variants were simultaneously present in varying proportions.[6] Based on chimpanzee studies performed by Dr. Farci,[7,8] it appears that neutralizing antibodies in HCV infection are isolate-specific rather than broadly reactive, and I presume that even had I developed an excellent neutralizing antibody response against my dominant clone, any other variant already present could have assumed dominance to maintain my infection. Indeed, Jens Bukh and collaborators[9,10] and also members of the McKeating laboratory[11] confirmed that I unequivocally developed neutralizing antibodies, but that they appeared late and were directed predominantly against the infecting strain and not against evolving variants. It became clear that for spontaneous clearance of NANBH/HCV, as occurs in approximately 20% of cases, the early immune response had to be sterilizing to prevent the more common scenario where the humoral immune response is always a step behind rapidly evolving strain heterogeneity. In parallel, Wedemeyer et al.[12] in the Rehermann laboratory demonstrated that T cell responses in my blood and that of other patients with NANBH virus infection were severely blunted in a

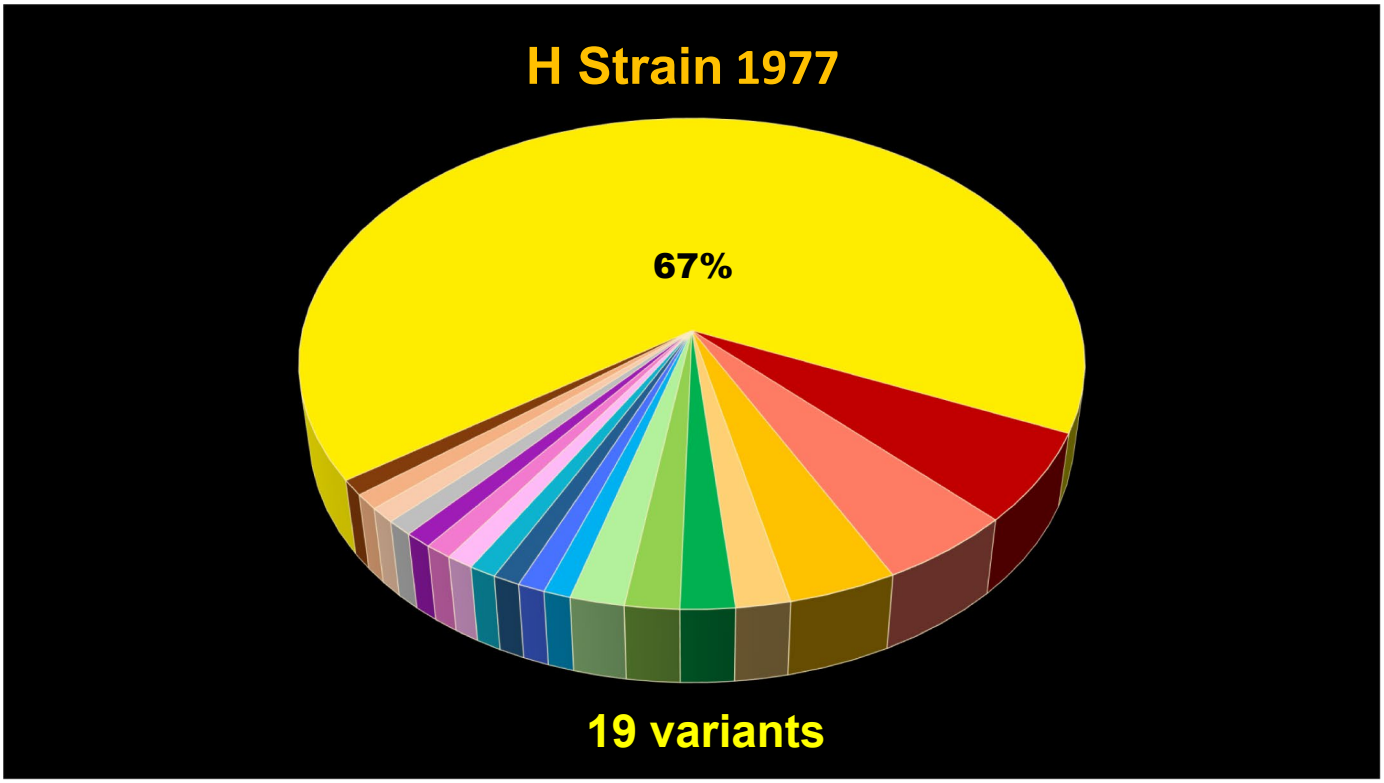

FIG 4 Quasi-species detected in the 1977 H strain infectious inoculum. One hundred four clones from the H77 sample were sequenced. Sixty-seven percent of the sequences were identical and represented the dominant strain. However, 18 other variants were simultaneously present, each with the potential to become dominant if the initial infectious strain was neutralized.

pattern later known as T cell exhaustion, while von Hahn et al.[13] showed that HCV continuously escapes from both neutralizing antibody and T cell responses during chronic infection. Further confounding the neutralization story, Pei Zhang et al.,[14] again using the H strain isolate, showed that the E2 envelope protein contained an adjacent antigenic site that was inhibitory to neutralization at HVR1. Thus, from a variety of experiments, it was evident that viral persistence was the outcome of a rapidly evolving viral quasispecies, a humoral response that could drive viral mutation but was insufficient to neutralize the viral strain *du jour* and a T cell response that was exhausted by combating a high antigenic burden that was fully entrenched before T cells could be effectively mobilized. Later studies measuring HCV viral load showed that my virus was almost completely contained early in infection, but was not eradicated such that a new variant assumed dominance and sequential persistence. Farci et al.[15] showed that this early containment in my case and others was predictive of a slow clinical evolution of chronic hepatitis C.

Discovering the exact nature of the NANBH agent in the days before the technologic advances of molecular biology proved more difficult than anticipated, and virtually every serological, biological, and early molecular assay utilized by NIH laboratories and many others throughout the world proved futile. Unbeknownst to the general scientific community, during the period 1981-1987, Michael Houghton, Qui Lim Choo, George Kuo, and others at Chiron Corporation in Emeryville, California, in collaboration with Daniel Bradley at the CDC, used pelleted plasma that was proved to be infectious in the chimpanzee, a GT-11 expression vector, and a presumed, but unproven, antibody from infected patients to clone the NANBH agent and rename it HCV.[16] This project was a Nobel Prize–worthy tour de force involving 6 years and millions of negative cloning experiments until a single reactive clone was finally identified. Subcloning, amplification, and "walking the genome" allowed definitive characterization of the agent as a flavivirus and identified antigenic sites sufficient to develop an immunoassay for detection of anti-HCV.[17] This assay identified pedigreed infectious sera and well-characterized negative controls with 100% accuracy in a coded NIH panel of blinded duplicates that 19 other laboratories claiming a NANBH virus assay had failed. In a critical confirmation of the assay, the Alter laboratory tested sera from 15 prospectively followed NANBH cases and found that every patient had seroconverted in close temporal relationship to the pattern of ALT elevations that defined their case definition.[18] Further, in 80% of 25 NANBH cases, a linked blood donor was found to be anti-HCV-positive by a first generation assay and 88% by a more sensitive second generation assay. The anti-HCV assay was mandated for blood screening in the United States in 1990 and by 1997, ongoing prospective studies at the NIH demonstrated the virtual eradication of transfusion-associated hepatitis, a dramatic decline from the 30% incidence rate documented in this same population in 1970. It can be estimated from prospectively defined incidence figures and projections to the entire transfused US population that as many as 4 million blood recipients might have been infected with hepatitis C in the decades of the 1970s and 1980s, when my story began. Conversely, millions more were protected from these infections after 1990. Do I wish that these tests had been in place for me in 1977? Not really, because I would have missed this whole adventure. I would have played no role in the conquest of HCV, and I would have died of my heart disease at age 92, just as I did.

Although my life is over, the HCV story is not. I'm still watching and excited to see the advent of nontoxic curative drugs with near 100% efficacy that have the potential to eliminate HCV on a global scale. The holy grail for global eradication will be development of a vaccine that will induce broadly reactive, genotype-independent neutralizing antibodies and/or potent T cell responses that will be sterilizing or, at least, significantly diminish progression to persistent viremia and chronic liver disease.[19]

As noted above, the results of many studies using my precious infectious plasma have shown that neutralizing antibodies can be induced, though they are thus far inadequate to clear natural infection in most individuals. Nonetheless, polyclonal immunoglobulin derived from my plasma has been shown to protect human liver chimeric mice from homologous challenge[20] and to passively protect chimpanzees from homologous, but not heterologous, challenge.[21] Further, in 1997, my strain H sequence was used to engineer the first infectious clones of HCV,[22,23] and this breakthrough contributed to the first HCV culture systems now of paramount importance for *in vitro* measurement of viral neutralization and ultimately to assess immunogenicity of vaccine candidates in the absence of a reliable and affordable small-animal model.

FIG 5 Patient H. Here I am blazing a mountain trail (left panel). And here I am in the apheresis unit on the occasion of my 90th birthday celebration (right panel). Front row: my wife, myself, and Cathy Schechterly (study coordinator). Standing: Barbara Rehermann, Harvey J. Alter, T. Jake Liang, Robert H. Purcell, Patrizia Farci, and Susan Leitman (Head of the Apheresis Center).

None of this could have been imagined in 1977, or perhaps even in 1997. I think back to a lot of what-ifs. What if my wife had not known how to do CPR? What if I had not had my heart surgery in a research hospital? What if I had not enrolled in a prospective study that collected and then intensively studied my infectious plasma? In probability, the HCV story would have ended the same way, but I like to think that my life accelerated the route to discovery. You may dispute this, but death has given me the ability to validate my life unblemished by mortal perspective and potential bias. I now have the moral high ground. This is my story and I'm sticking to it.

Respectfully,
Mr. H (Fig. 5)

SERIES EDITOR'S POSTSCRIPT

When I first approached Harvey Alter (Lasker Awardee in 2000 and coauthor of the initial publications on the discovery of hepatitis B as well as HCV) and invited him to relate the story of HCV for the current series of historical essays, he hesitated because he said that he had written all he had to say about HCV. But shortly thereafter he relented and told me that he had a new angle on the topic that was too secret to reveal, even to the Series Editor. And here you have the finished product. Who else but the inimitable Harvey would have contrived to tell the HCV biography in the voice of one of his earliest and now-deceased patients with HCV infection, who donated his plasma to HCV research and after whom an HCV strain (strain H) was named. Here, indeed, we have the ultimate stepwise account of the discovery of HCV and the intimate unfolding of its identification and history, by a skilled raconteur who was himself part of the story from step one.

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REFERENCES

1) Feinstone JM, Mihalik KB, Kamimura J, et al. Inactivaton of hepatitis B virus and non-A, non-B virus by chloroform. Infect Immun 1983;4:816-821.

2) He L-F, Alling DW, Popkin TJ, et al. Determining the size of non-A, non-B hepatitis virus by filtration. J Infect Dis 1987;156:636-640.

3) Shimizu YK, Feinstone SM, Purcell RH, et al. Non-A, non-B hepatitis: Ultrastructural evidence for two agents in experimentally infected chimpanzees. Science 1979;205:197-200.

4) Berman MD, Alter HJ, Ishak KG, et al. The chronic sequelae of non-A, non-B hepatitis. Ann Intern Med 1979;91:1-6.

5) Ogata N, Alter HJ, Miller RH, et al. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. Proc Natl Acad Sci USA 1991;15:3392-3396.

6) Farci P, Purcell RH. Clinical significance of hepatitis C virus genotypes and quasispecies. Semin Liver Dis 2000;20:103-126.

7) Farci P, Alter HJ, Wong DC, et al. Prevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. Proc Natl Acad Sci USA 1994;91:7792-7796.

8) Farci P, Shimoda A, Wong D, et al. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervartiable region 1 of the envelope 2 protein. Proc Natl Acad Sci USA 1996;93:15394-15399.

9) Meunier J-C, Engle RE, Faulk K, et al. Evidence for cross-genotype neutralization of hepatitis C virus pseudo particles and enhancement of infectivity by apolipoprotein C1. Proc Natl Acad Sci USA 2005;102:4560-4565.

10) Meuleman P, Bukh J, Farhoudi A, et al. In vivo evaluation of the cross-genotype neutralizing activity of polyclonal antibodies against hepatitis C virus. Hepatology 2011;53:755-762.

11) Logvinoff C, Major ME, Oldach D, et al. Neutralizing antibody response during acute and chronic hepatitis C virus infection. Proc Natl Acad Sci USA 2004;101:10149-10154.

12) Wedemeyer H, He S-S, Nascimbeni M, et al. Association and effector function of hepatitis C virus specific CD4+ and CD8+ T cell responses in long-term recovered and chronically infected patients. J Immunol 2002;169:3447-3458.

13) von Hahn T, Yoon JC, Alter H, et al. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. Gastroenterology 2007;132:667-678.

14) Zhang P, Zhang L, Struble EB, et al. Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. Proc Natl Acad Sci USA 2009;106:7537-7541.

15) Farci P, Wollenberg K, Diaz G, et al. Profibrinogenic chemokines and viral evolution predict rapid progression of hepatitis C to cirrhosis. Proc Natl Acad Sci USA 2012;109:14562-14567.

16) Choo Q-L, Kuo G, Weiner AJ, et al. Isolation of a c-DNA clone derived from a blood-borne non-A, non-B hepatitis genome. Science 1989;244:359-362.

17) Kuo G, Choo Q, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244:362-364.

18) Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. New Engl J Med 1989;321:1494-1500.

19) Bartenschlager R, Baumert TF, Bukh J, et al. Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies. Virus Res 2018;248:53-62.

20) Vanwolleghem T, Bukh J, Meuleman P, et al. Polyclonal immunoglobulins from a chronic hepatitis C virus patient protect human liver chimeric mice with infection from homologous but not heterologous challenge. Hepatology 2008;47:1846-1855.

21) Bukh J, Engle RE, Faulk K, et al. Immunoglobulin with high-titer cross neutralizing hepatitis C virus antibodies passively protects chimpanzees from homologous, but not heterologous challenge. J Virol 2015;89:1-5.

22) Yanagi M, Purcell RH, Emerson SU, et al. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. Proc Natl Acad Sci USA 1997;94:8738-8743.

23) Kolykhalov AA, Agapov EV, Blight KJ, et al. Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA. Science 1977;277:570-574.