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Michael J. Sofia *Editor*

HCV: The Journey from Discovery to a Cure

Volume I

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31
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Michael J. Sofia
Editor

HCV: The Journey from Discovery to a Cure

Volume I

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Preface

The story of how the battle against hepatitis C was won began in 1975 with the realization that a previously unknown virus, non-A non-B hepatitis (NANBH), was responsible for a liver disease that plagued millions of individuals worldwide and took the lives of hundreds of thousands. It wasn't until the efforts of Harvey Alter, Michael Houghton, and their collaborators that in 1989 the hepatitis C virus (HCV) was identified as the new virus. Through their efforts, the development of a way to screen the blood supply was achieved and the risk of contracting this disease was dramatically reduced. However, there were still tens of millions of individuals who remained infected and transmitting the disease either sexually, via IV drug use or by coming in contact with contaminated blood by other means. The need for a cure was critical. This two-volume book attempts to chronicle the scientific story of the discovery of the virus, the development of tools important to the search for a cure, and the many drug discovery and development efforts that eventually delivered curative therapies to the millions of chronically infected HCV patients. It also attempts to put context around the impact of this work for the patient and society.

In conceiving this book, *HCV: The Journey from Discovery to a Cure*, I wanted to not simply have a series of isolated accounts of drug discovery efforts that led to marketed products. I wanted to take the reader along the entire historical scientific journey from the beginning to the end. It is rare in the annals of science that within a lifetime the full story of the identification of the key causative agent for a disease is found, and a cure is identified and made available to patients. In fact, cures of diseases are extremely rare, and the cure for HCV is the only example of a cure for a chronic viral disease. Therefore, I felt that the entire story needed to be told in one place.

In this two-volume account of how an HCV cure was achieved, the journey is communicated by those scientists and clinicians, including five Lasker Award Laureates, who were making those critical contributions integral in making this achievement happen. It begins with accounts of the discovery of the virus, elucidation of the virus life cycle and the role of each viral protein, development of the replicon system, and the use of interferon as early therapy. It continues with sections

focused on each of the key viral drug discovery targets. Each of these drug discovery sections first provides a general overview of the evolution of medicinal chemistry efforts against the target followed by detailed accounts of the discovery of each drug that is now a marketed HCV therapy. Yet the account of how HCV was cured would not be complete without addressing the evolution of innovative clinical trials and how combination therapies evolved to deliver therapies that are now pan-genotypic, provide exceptionally high cure rates in 8–12 weeks, and exhibit high barriers to resistance. Finally, the true indicator of medical achievement is not the commercial launch of a drug but the benefits that medicines bring to the patient and society; therefore, Volume 2 of the book ends with several perspectives speaking to the benefits achieved by an HCV cure and the possibilities for eliminating HCV as a global health threat.

What this two-volume book does not attempt to do is capture the vast body of work that was published over the 24 years that spanned the time from identification of the virus in 1989 to the approval of the first interferon-free HCV cure, sofosbuvir, in 2013. It also doesn't attempt to capture in detail the stories of the many failed avenues of investigation or accounts of the many investigational drugs that never made it to regulatory approval. However, this book does capture what I feel are the seminal contributions to the field and the important drug discovery success stories that matter to patients.

Finally, I have to thank all the chapter authors who committed a great deal of time outside of their busy schedules to tell the stories contained in this book. Each of them made their contribution because they too saw the need to tell the full story and wanted to be part of it. I also must thank all those researchers and clinicians who have contributed to the HCV cure story over 24 years but whose names are not explicitly mentioned in this work. Your contributions are not lost on those who have authored these chapters.

Warminster, PA, USA Michael J. Sofia

Contents

Part I
The Virus & Early Therapy

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From B to Non-B to C: The Hepatitis C Virus in Historical Perspective

Harvey J. Alter

Contents

Abstract The discovery of HCV was an evolutionary process beginning with the serendipitous identification of the Australia antigen that later proved to be the surface protein of the hepatitis B virus (HBV) and the first marker for any human hepatitis virus. Studies of transfusion-associated hepatitis made it evident that most cases were unrelated to HBV. The later discovery of the hepatitis A virus (HAV) made it apparent that non-B cases were also non-A leading to the awkward terminology non-A, non-B hepatitis (NANBH). While NANBH was identified only by exclusion and had no specific serologic or molecular marker, using chimpanzee transmission studies, it was possible to show that the NANBH agent was small and enveloped and most consistent with being a flavivirus as it later proved to be. Clinical studies showed that NANBH could lead to cirrhosis, hepatocellular carcinoma, and liver-related fatality. The major breakthrough occurred in the late 1980s when the Chiron Corporation cloned the NANBH agent and renamed it the hepatitis C virus (HCV). Adding HCV serologic testing, and later molecular testing, to routine donor screening virtually eradicated TAH with current risk estimated to be one case in two million transfusions. More recently, direct-acting antiviral agents have been shown to result in sustained virologic responses, tantamount to cure, in 98% of treated subjects. The existing challenges are to identify currently unrecognized HCV carriers and to make treatment accessible to all.

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Preface

This chapter is not intended to be a comprehensive review of the evolution of HCV but rather a personal vignette of how I saw the story from my vantage point at the NIH where much of the early story played out. The HCV story is historically and integrally related to the HBV story in that only by the ability to serologically or molecularly exclude HBV, and later HAV, was non-A, non-B hepatitis (NANBH) identified, setting the stage for the subsequent cloning of the NANBH agent and its renaming as HCV. I apologize for many omissions in this narrative, but the essential early history of HBV and HCV is chronicled herein, and perhaps the story is best told by one old enough to have been there. Sadly, many of the key early players are now deceased including Baruch Blumberg, Thomas London, Irving Millman, JS Dane, Palmer Beasley, Wolf Szmuness, Saul Krugman, and Hans Popper. I dedicate this chapter to their memory.

1 The Era of Observation Prior to Testing

Clinical observation, recurrent war, and serendipity have forged the approximate 2,000-year history of viral hepatitis. The original descriptions of hepatitis have been attributed to Hippocrates circa 425 BCE. He observed yellowing of the skin that he termed "ikterus" and hardening of the liver that he termed "kirros." These observations antedated the germ theory of disease and could have had diverse etiologies but most likely were cases of hepatitis A and B related to contaminated water supplies and medical practices that involved percutaneous exposure with shared devices. The ancient nature of hepatitis viruses is also confirmed by the finding of HBV sequences in a sixteenth-century Korean mummy [1]. In the seventeenth and eighteenth centuries, many outbreaks of parenterally transmitted hepatitis were traced to contaminated vaccines for syphilis, smallpox, and other infectious agents of the time. Generally the vaccines were fortified with human plasma that was later proved to be contaminated, as in a well-documented outbreak among vaccinated shipyard workers in Bremen Germany in 1885 [2]. In essence, medical history from Hippocrates forward was marked by wartime outbreaks of hepatitis that became known as campaign jaundice and by injection-transmitted hepatitis traced to therapeutic or preventive measures that utilized pooled human plasma. A very well-characterized vaccine-induced outbreak was traced to contaminated lots of yellow fever vaccine during World War II [3]; 50,000 soldiers developed clinical hepatitis, and undoubtedly many fold that number were asymptomatically infected. Retrospective seroepidemiology showed these cases to be caused by HBV [4]. The frequent and debilitating role of hepatitis during wartime fostered many studies primarily supported by the army. These studies clearly distinguished a short incubation, highly

infectious, and generally self-limited form of hepatitis that was designated hepatitis A and a more protracted form that was parenterally transmitted and designated hepatitis B [5]. These were superb epidemiologic studies but failed to identify either a causative agent or develop a specific assay for their detection. Early human volunteer studies added important pieces to the transmission of these two forms of hepatitis and to clinical outcomes but have come under ethical criticism because they employed prisoners or institutionalize children [6, 7]. These studies could not be performed under current patient protection mandates, but they did have institutional approval and participant or parent consent at the time they were performed. Studies performed at the Division of Biologic Standards, the forerunner of the FDA, utilized dilutions of hepatitis B infectious sera and defined the minimum infectious dose and the incubation period as related to dose showing that more diluted inocula had a longer incubation period [6]. Studies at the Willowbrook State School in NJ confirmed two epidemiologic forms of hepatitis and importantly identified a single patient (MS) who sequentially developed a short incubation form of hepatitis, designated MS-1, and a long incubation form, designated MS-2 [7]; these temporal characteristics were maintained when passaged to other study subjects. Years later when the Australia antigen was identified, it was shown that MS-1 was Australia antigen negative and MS-2 was Australia antigen positive and hence HBV related. It was also shown that MS-2 could be inactivated by boiling and induce protective antibody when fed to naïve subjects [8]. This was the first proof of principal for a hepatitis B vaccine.

Despite these many studies, the viruses that caused hepatitis A and B were never observed, and no test for their presence was developed. Diagnosis depended solely on epidemiology, clinical observation, and elevations of serum transaminase in temporal relationship to clinical presentation. This all changed by the serendipitous finding of the Australia antigen in the early 1960s [9] and the linking of Australia antigen to cases of hepatitis in 1967 [10].

2 The Australia Antigen Transformation

In the late 1950s and early 1960s, Baruch Blumberg, a geneticist working at NIH, performed studies that focused on protein polymorphisms in human subjects. He discovered polymorphic variations in beta-lipoproteins that were detected by Ouchterlony agar gel diffusion. In these studies, serum from a multiply transfused patient was arrayed against normal sera in a 7-well agar diffusion pattern. The underlying presumption was that multiply transfused subjects might develop antibodies against normal serum components antigenically different than their own. When these antibodies diffused into the agar, they would react with distinct and specific antigens diffusing in the opposite direction. The antigen-antibody reaction was characterized by a curved precipitin line that was then overlaid with a lipid stain (Sudan Black). A blue precipitin band was indicative of a lipoprotein polymorphism. Against this background, Harvey Alter was at NIH at the same time and using similar

Ouchterlony technology to search for protein differences that might be the cause of febrile or other transfusion-associated reactions. Dick Aster, also working in the NIH Blood Bank at that time, heard a lecture by Blumberg and reported back on the similarity between these research approaches. Within days, a collaboration was formed between the Blumberg and the Alter labs. The first element of serendipity in this story struck when Alter, while looking for additional beta-lipoprotein polymorphisms, detected a precipitin line that did not take up the lipid stain but rather turned red when a protein counterstain, azocarmine, was applied to the agar plate. This unique precipitin was found to represent an immune reaction between the serum of a multiply transfused hemophiliac and an Australian aborigine. The aborigines were one of many global populations whose sera were stored in Blumberg's serum bank for genetic studies. The significance of this finding, if indeed there was any, was unknown at the time. For a brief period, the unique antigen was called the "red antigen" for its staining characteristics, but then debate ensued as to whether to call it the Bethesda antigen for the place where it was discovered or the Australia antigen for the person in whom it was discovered. Blumberg decided on the latter terminology because it was consistent with the nomenclature for newly discovered hemoglobin polymorphisms; hence, the birth of the Australia antigen (Au).

In the next phase of investigations, we studied the prevalence of Au in healthy subjects and various NIH patient populations. Strikingly, it was found in only 0.1% of healthy donors, and in under 1% of most diseases tested, but in 10% of patients with leukemia. The first publication of this finding [9] stressed the association with leukemia and even speculated that this might be an antigen on a then postulated leukemia virus or represent the product of a gene that induced susceptibility to leukemia. There was no hint at that time (1965) that there was any relationship to hepatitis, but in retrospect the high prevalence in patients with leukemia reflected their high exposure to blood transfusion and their immune deficiency state that prevented clearance of the virus once infected. At this point in the story, Blumberg left NIH to head a research division at the Institute for Cancer Research in Fox Chase, Philadelphia. The rest of this early hepatitis virus history played out in Philadelphia. Blumberg, who was a geneticist and had a penchant for hypothesizing, believed that Au was genetically determined and predisposed individuals to leukemia. He thus initiated studies to test patients who had a known genetic predisposition to develop leukemia, namely, patients with Down's syndrome. In collaboration with Alton Sutnick and the late Tom London, institutionalized patients with Down's syndrome were tested, and strikingly, near 30% were found Au positive [11]. Although this supported the genetic hypothesis, much to the investigators' credit, they performed additional studies of Down's patients in different environmental settings. While the prevalence in large institutions for the mentally retarded approximated 30%, it was only 10% in smaller institutions, zero among Down's patients living at home and zero in newborns with Down's. This was inconsistent with the genetic hypothesis and the first clue that Au might be associated with an infectious disease. Thus, a failed hypothesis serendipitously led to a major insight in the Au story, namely, its link to an infectious disease. The third serendipitous event was that a technologist in the Blumberg lab, Barbara Werner, who had always served

as the Au-negative control came to work with classic symptoms of hepatitis and tested her own blood to find that she had seroconverted to Au positivity. This was a eureka moment that tied the Au story together and strongly suggested that Au was integrally linked to a hepatitis virus [10]. This was easily confirmed in subsequent studies of patients in various settings with and without hepatitis [11]. Subsequently, the late Fred Prince at the New York Blood Center confirmed the relationship to hepatitis and showed that Au was specifically linked to serum hepatitis (hepatitis B) and not to epidemic hepatitis A [12]. This led to a series of nomenclature revisions whereupon Au was sequentially called the SH antigen for serum hepatitis, the hepatitis-associated antigen (HAA), and finally the hepatitis B surface antigen (HBsAg) after it was shown to be a component of the viral envelop and not of other components of the virion. Studies by Bayer et al. in the Blumberg lab [13], Gerin and Purcell at NIH [14], and Dane at the Royal Free Hospital in London [15] showed that HBsAg was distributed on small circular and tubular particles that were in great abundance as compared to the whole virion which came to be known as the Dane particle. It was the great excess of non-virion antigen that made this agent detectable by a technique as insensitive as agar gel diffusion.

With the sudden availability of a serum marker for HBV, studies by Okochi in Japan [16] and Gocke in the USA [17, 18] unequivocally linked the Australia antigen to posttransfusion hepatitis B. Other studies used this new assay to measure prevalence and incidence among healthy and diseased populations throughout the world, to test disease associations, and to assess interventions to control or eradicate HBV transmission. Three studies are of particular note. In 1980, Szmuness et al. [19] tested the clinical efficacy of a plasma-derived HBV subunit vaccine developed by Maurice Hilleman at Merck. This vaccine was evaluated in 1,083 gay men in New York City where half received vaccine and half served as controls. Greater than 95% of susceptible vaccinees developed protective antibody to HBsAg, and the hepatitis attack rate in vaccine recipients was 3.2% compared to 25.6% in controls ($p < 0.0001$). Vaccine failures were presumed due to high-risk enrollees who were already incubating HBV infection at the time of vaccination. Indeed, in those who received the full course of the three-dose vaccine, efficacy was 100%. Although this vaccine had low uptake in the population because of fear of the then emerging AIDS epidemic, it served as proof that an HBV vaccine could be highly efficacious and stimulated the development of a recombinant vaccine that has become a universal vaccine in the USA and much of the developed and developing world. A second study of particular note was conducted by the late Palmer Beasley and coworkers in Taiwan [20] wherein they tested the value of hepatitis B immune globulin (HBIG) and the serum-derived HBV vaccine to prevent neonatal HBV transmission from highly infectious HBV e-antigen-positive mothers. In the absence of HBIG or vaccine, 90% of offspring were HBV infected and became HBsAg chronic carriers. In those who received three doses of HBIG alone or vaccine alone, the carrier rate was reduced to 23%, and in those who received the combination of HBIG and vaccine, only 5% became carriers; the 5% failure rate was presumed due to intra-uterine infection. The third critical study was also conducted by Beasley and coworkers [21] wherein they enrolled 22,707 Taiwanese government workers and

investigated the cause of death in those who were HBsAg positive or negative at the onset of the study. There were 105 deaths in the HBsAg-positive cohort of which 40 (38%) were attributed to hepatocellular carcinoma (HCC) and 17 (16%) to cirrhosis. In contrast, there were 202 deaths in the much larger cohort of HBsAg-negative subjects among whom only 1 died of HCC and 2 of cirrhosis. The relative risk of these fatal hepatitis B events was greater than 200-fold higher in those who were HBsAg positive. Michael Kew performed meticulous and comprehensive studies in Africa that confirmed the strong association between HBV infection and HCC [22].

One cannot overestimate the importance of the Australia antigen discovery as it led to the first diagnostic test for any hepatitis agent, the first specific viral marker for screening blood donors that dramatically reduced HBV transmission by blood transfusion, the first means to screen pregnant women and interdict perinatal HBV infection, and the basis for a first-generation hepatitis B vaccine that was 95% protective in clinical trials [16]. HBsAg also served as a link and predictive marker of hepatocellular carcinoma, the most prevalent form of liver cancer in Africa and Asia. The ability to prevent HBV infection with a virus-specific vaccine made this, in essence, the first cancer vaccine.

3 The Advent and Ascent of Non-A, Non-B Hepatitis: The Blood Transfusion Story

In 1965, Paul Holland, Paul Schmidt, and Bob Purcell initiated a prospective study of transfusion-associated hepatitis (TAH) among open heart surgery patients at the NIH Clinical Center. I became chief investigator in that study in 1970, and it has continued with some modifications to the present time. The basic study design was to obtain and store samples pre-transfusion and then every 1–2 weeks posttransfusion for 3 months followed by monthly samples for an additional 3 months. The retention of linked donor samples was added later. Since no specific viral markers were available at that time, hepatitis was diagnosed solely on the basis of ALT elevations that exceed 2.5 times the upper limit of normal (ULN) from 2 to 26 weeks posttransfusion followed by a second weekly sample that exceeded 2.0 times ULN. Patients with such elevations had additional sampling and were generally followed long-term.

The first major finding from these studies was that prior to 1970, open heart surgery patients at NIH had an astounding 30% incidence of TAH. This inordinate number was predicated on three factors, namely, the high volume of blood received (average 17 units per procedure), the prospective nature of the study which detected anicteric cases that would not have otherwise been reported, and the use of paid-donor blood. In an early and definitive analysis of this population, Walsh et al. [23] showed that patients who received at least one unit of paid-donor blood had a 51% incidence of TAH compared to only 7% in those who received only volunteer donor

blood. In 1970, based on the Walsh study and earlier published studies by John Allen [24], we adopted an all-volunteer donor system. Simultaneously, we initiated screening of blood donors for HBsAg using an unlicensed agar gel diffusion technology identical to that used in the discovery of the Australia antigen. This combined approach resulted in a dramatic 70% reduction in TAH incidence [25]. Indeed, no subsequent intervention has had as much impact on TAH, since the incidence was so high at the time. Although two variables were introduced at the same time, we could deduce that the primary factor in the massive reduction in hepatitis incidence was the introduction of an all-volunteer donor system. The source of blood proved to be the dominant factor in blood safety.

Assays for HBsAg became increasingly sensitive, and by 1973, a licensed enzyme immunoassay (EIA) was used to test stored samples from the periods before and after HBsAg screening. This revealed that even before HBsAg donor screening, no more than 30% of TAH was due to the hepatitis B virus [25], leaving open the causation of the observed non-B cases. In 1973, Feinstone, Kapikian, and Purcell at NIH [26] discovered the hepatitis A virus (HAV) using immune electron microscopy to test acute and chronic phase samples from an outbreak of hepatitis A. This method, while tedious, was accurate and sensitive, and we immediately sent samples from our non-B hepatitis cases to the Feinstone lab. Not a single non-B case was found to be HAV infected coincident with transfusion, suggesting that the main cause of TAH was a previously unknown virus or group of viruses. This resulted in the first publication to describe this previously unrecognized agent of transfusion-transmitted hepatitis [27]. We gave this new entity the nondescript name, non-A, non-B hepatitis (NANBH) rather than hepatitis C virus because we had not yet proven its viral nature nor the number of agents that might be involved.

Two important elements helped elucidate the nature of NANBH. First were transmission studies in chimpanzees. Inoculating samples from patients with acute and chronic NANBH and from implicated donors, the Alter and Purcell labs were able to routinely transmit infection to chimpanzees and then serially passage the infection in this model [28]. Tabor and coworkers at FDA [29], in parallel studies, also transmitted NANBH to chimpanzees. Infected chimpanzees were asymptomatic but developed ALT elevations in a pattern that closely mimicked human infection, and liver biopsies confirmed histologic evidence of hepatitis. Having this model, we were then fortunate to obtain an apheresis collection early in NANBH infection from a patient (WH) who was developing a severe case of icteric TAH. The Purcell lab then made dilutions of the WH plasma unit and used these to perform titration studies in chimpanzees. It was determined that WH plasma had an infectivity titer of $10^{6.5}$ chimpanzee infectious doses per milliliter. Interestingly, a decade later when PCR became available, we showed that the HCV titer of the WH sample was 10^7 and hence almost identical to the infectious titer in chimps. Having the titered NANB inoculum and the chimpanzee model allowed for further studies to characterize the agent. Steve Feinstone performed chloroform extraction of the WH inoculum, and in a carefully controlled study [30] that included a mock extraction, he was able to show that chloroform abrogated infectivity in the chimp indicating that the agent was enveloped and contained essential lipid in its membrane. Subsequently, Li-Fang He

in the Purcell lab [31] performed filtration studies wherein WH plasma was placed on filters of varying sizes and the filtrates tested for infectivity in the chimpanzee. This showed that the agent passed through a 50 nm filter, but not through a 30 nm filter. Thus, even before the agent was observed and before there was either an antigen or antibody test for specific identification, it could be deduced that the NANBH agent was small and lipid encapsulated. This narrowed the field of potential viral classes and, having ruled out any relation to HBV, was most consistent with NANBH being a small RNA virus in the alpha or flavivirus family or representing an entirely new class of viral agents. It is my recollection that Daniel Bradley at CDC was the first to suggest that the data were most consistent with the agent being a flavivirus, as it proved to be.

Not only was the precise nature of the virus obscure, but so too was its clinical significance. Because most cases were asymptomatic and identified only through prospective studies and since enzyme elevations in those cases were generally modest, there were some who considered NANBH a simple transaminitis of minor significance. The benignity of the disease was dispelled by a study conducted by the Clinical Center Liver Service. In this study, Berman et al. [32] performed liver biopsy on 39 patients with NANBH, most derived from our prospective study of TAH. While the majority of these patients had only mild to moderate hepatitis without significant fibrosis, 10% already had cirrhosis when first biopsied, and an additional 13% had what was then called chronic active hepatitis (CAH). When 20 patients were re-biopsied several years later, 5 more cases of cirrhosis had evolved such that the total with cirrhosis was 8 of 39 or 20%. Moreover, three of those with cirrhosis died of their liver disease and three others died of their underlying heart disease but also had severe liver disease as a cofounding event. This study and many others to follow [33–35] established NANBH as a generally mild disease but one that could progress to cirrhosis and ultimate fatality. Interestingly, the 20% rate of progression to cirrhosis has held up over the years, although current estimates with longer duration of follow-up are closer to 30–40%. Because of the vast number of persons with chronic HCV infection, estimated to be 350 million globally and 3 to 5 million in the USA, the disease burden is enormous, even if only 30–40% progress to dire outcomes. In the USA, HCV is the most common hepatitis infection, the most prevalent cause of end-stage liver disease, and the leading indication for liver transplantation. Despite the urgent need for a diagnostic and therapeutic breakthrough, up through the late 1980s, we remained without a specific diagnostic assay, without a visualized agent, and without therapy for chronic NANBH except for interferon, an arduous and toxic therapy that was only 10–20% effective at that time. All this changed with the unexpected cloning of the NANBH/HCV agent in 1989 [36] and the subsequent development of sensitive serologic and molecular assays and the evolution of drugs that sequentially have seen sustained virologic response rates, tantamount to cure, that exceed 95%.

4 The Cloning of HCV

From 1975, when NANBH was first identified as a clinical entity, through 1989 when the agent was cloned by Houghton and coworkers at Chiron [36], multiple efforts in the USA, Europe, and Japan to identify a specific antigen or antibody and hence to develop a serologic assay were unsuccessful, demonstrating how difficult it is to identify one component of a serologic reaction in the absence of the other. Further, molecular detection was not possible until the advent of PCR and advances in molecular technology and, as we later learned, was further limited by the generally low titer of the NANBH agent. In the 1980s, molecular biology was in its infancy, and techniques that are simple and routine today were state of the art at that time. The quest for the NANBH agent was a black box, a seemingly insoluble equation because none of the variables were known. From 1975 to 1985, multiple laboratories in the USA, Europe, and Japan attacked this conundrum, but none were able to solve the riddle as evidenced by failure to break the code on a well-characterized NANBH panel. Into this void entered the Chiron Corporation that unbeknownst to the general hepatitis community worked for 6 years to blindly clone the NANB agent. The Chiron team led by Michael Houghton, Qui-Lim Choo, George Kuo, and Amy Weiner in collaboration with Daniel Bradley at CDC embarked on this arduous path to discovery that hinged on three critical elements. First, Bradley, in meticulously designed studies, extensively characterized infectious NANBH inocula in the chimpanzee model [37, 38]. These samples were titered, serially passaged, collected in relatively large volumes, and made available to Chiron for their cloning studies. The starting point was to pellet the source material, extract RNA, and then reverse transcribe to cDNA. The second key element of the study was to use a phage gt-11 expression vector such that genetic information in cDNA fragments would be expressed as protein when the phage infected *E. coli*. The third critical element of study design was to assume that patients with resolved or chronic NANBH would have circulating antibody to the virus even though no such antibody had been detected in the preceding decade. The fourth key element of this endeavor was perseverance, because success was slow in coming, and corporate leadership began to question the soundness of their investment. The Houghton team persisted despite sometimes demoralizing setbacks, and it has been said by the late Lacy Overby, another collaborator, that six million clones were screened with presumed antibody before there was a single positive reaction. This single reactive clone was then subcloned and the contained nucleic acid sequenced. A short genomic segment was characterized, and subsequent cloning allowed the investigators to "walk" the genome and express an antigen that could serve as a target for antibody screening [39]. A prototype antibody assay was developed, principally by Kuo [40], and was ready to be challenged by the NANBH panel held at the Alter lab. This small panel consisted of NANBH samples from patients and donors that had proven infectious in the chimpanzee and control samples from healthy donors whose blood had been transfused to at least ten recipients without being implicated in hepatitis transmission. Importantly, all samples in the panel were present in duplicate, and duplicates

were placed in random positions. Although 19 other purported NANB virus assays had failed this panel, the Chiron assay detected antibody in all known chronic NANBH carriers and, importantly, failed to detect antibody in 14 samples from 7 pedigreed donor controls. Samples from two patients with acute NANBH were missed, but that was because antibody had not yet developed; subsequent samples from these patients demonstrated antibody seroconversion. Thus, in this small but difficult panel, the Chiron assay had perfect sensitivity and specificity. The specificity of the assay for the agent of NANBH was further validated by testing cases and controls in the NIH prospective cohort wherein it became clear that this assay had it been applied to routine donor screening could have prevented 80% of TAH [41]. Analysis of a second-generation antibody assay introduced in 1992 predicted the prevention of 88% of TAH cases in the NIH cohort. Hence, 1990 introduced the first specific assay for the NANBH agent and a suitable test for blood donor screening, as well as a diagnostic assay to evaluate hepatitis cases and to screen populations. The non-A, non-B virus was renamed the hepatitis C virus (HCV) and 1990 ushered in the age of HCV.

Following this breakthrough, Chiron investigators sequenced the entire genome and showed that it coded for a polypeptide that was then posttranslationally cleaved into structural and nonstructural units. Using other flaviviruses as a model, they and other investigators identified genomic regions with enzymatic functions critical to viral replication [39]. These regions, including NS3, NS4, NS5A, and NS5B, have become targets of highly efficacious inhibitors that completely block viral replication as will be described in subsequent chapters of this book.

5 The Virtual Eradication of Transfusion-Associated Hepatitis

As described above, the most important measure in the prevention of TAH was the prohibition of paid-donor blood [23]. Those who sold their blood, particularly prior to 1970, often came from derelict populations where abusive alcohol and drug use and other high-risk behaviors were prevalent. Further, such individuals donated as frequently as possible, which could be weekly if they were selling plasma rather than whole blood. Hence, a single hepatitis-carrier donor could inflict major damage to multiple recipients with no viral screening assays in place to interdict this practice. As indicated above, the prohibition of paid donors at the NIH Clinical Center in 1970 was primarily responsible for the dramatic decline in TAH incidence from 30 to 10% [25]. By 1971, all US blood establishments adopted an all-volunteer donor system under mandate from the FDA. In 1973, improved enzyme immunoassays for HBsAg entered the market and caused a further decline in HBV transmission to very low levels. However, the absence of a test for non-A, non-B hepatitis kept total TAH incidence at around 6% in the NIH prospective study [42]. A retrospective analyses of the NIH cohort predicted that ALT testing of blood donors might affect a 30%

decline in hepatitis transmission [43]. Similar predictions came from a multicenter prospective study (Transfusion-Transmitted Virus Study, TTVS) that was supported by the National Heart Lung and Blood Institute (NHLBI) [44]. Hence, in 1981, we and a few other hospitals introduced routine donor ALT testing, but disappointingly ongoing studies did not show a benefit of such testing. We next anticipated that testing for HIV, which was introduced in 1985, would indirectly reduce TAH incidence because HIV-infected patients were frequently coinfected with hepatitis viruses. We did not observe the anticipated collateral benefit of HIV testing because HBV blood transmission was already well controlled and because the primary victims of the early HIV epidemic were men who had sex with men and NANBH was not prevalent in that population until sexual promiscuity was later compounded by intravenous drug use. It was the use of shared needles that gave rise to the HCV epidemic, not promiscuous sexual activity. Thus by 1986, TAH incidence continued to hover around 6%. At that time another retrospective analysis by the TTVS and NIH groups [45, 46] indicated that using antibody to hepatitis B core antigen (anti-HBc) as a surrogate for NANBH might affect a 30–40% reduction in TAH. Indeed that was the case when we introduced this test as a donor screen in 1986 and when it was FDA mandated in 1987. After anti-HBc screening, TAH incidence in the NIH prospective study fell to 4%. On that background, HCV was cloned in 1989 and a first-generation commercial anti-HCV assay introduced into blood screening in 1990; an improved second-generation assay was introduced in 1992. It was routine HCV testing that broke through the resistant wall of TAH, and by 1997, the NIH prospective study demonstrated the virtual eradication of TAH [42]. We saw no further cases of HCV transmission and no cases of the then postulated non-A, non-B, non-C hepatitis. While the NIH prospective study is too small to claim eradication, the extent of transfusion transmission is now so low that it has to be estimated mathematically. It has been estimated that the current risk of transfusion-transmitted HCV is approximately 1:2,000,000 [47]. HBV transmission has also been dramatically reduced. It is my current contention that there is no specific non-A, non-B, non-C hepatitis agent and that those reported cases represented surgery-related inflammation, drug toxicity, or nonalcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH). Hence, in three decades, TAH incidence declined from approximately 30% to virtual zero, a triumph of a series of donor interventions and the introduction of increasingly specific and sensitive assays for HBV and HCV. This triumph is another important piece of the HCV story.

6 The End of the Beginning and the Beginning of the End

We are clearly at the “end of the beginning” of the HCV saga. Transfusion-associated hepatitis has been virtually eradicated; excellent population screening assays are in place as are sensitive methods to detect HCV RNA and to separate chronic carriers from those who spontaneously or therapeutically cleared the infection. As described in this book, amazing therapies have been developed that could

not have been imagined only a decade ago. It is now possible to cure 98% of HCV carriers, even if they have already progressed to cirrhosis [48]. Viral clearance has been shown to halt fibrosis progression and, in some cases, to induce fibrosis regression [49]. In those who achieve a sustained virologic response that is tantamount to cure, progression to hepatocellular carcinoma is markedly diminished [50], though not totally prevented because cancerous transformation had already been initiated at the time of virologic cure. In only four decades since the first recognition of non-A, non-B hepatitis, it is possible to envision the near eradication of HCV infection even in the absence of an HCV vaccine. In theory, from this day forward, no one identified with HCV infection should succumb to its long-term sequelae of cirrhosis, HCC, and end-stage liver disease. However, this optimistic projection is marred by several impediments to eradication. First, the CDC estimates that only half of HCV-infected individuals are aware of their infection, and hence there is a vast pool of infected individuals who might never be treated or who might not be identified until they present with severe liver disease. Eradication of HCV will depend on large-scale antibody screening of all populations at high risk and possibly even those without identified risk. The CDC has identified the birth cohort of 1945–1965, the Baby Boomers, as a fertile source to detect silent carriers, but this is not sufficient to detect the majority of infected individuals. I would suggest that every person seeking medical care in emergency rooms, outpatient clinics, hospital admissions or through their personal physicians of any specialty should have their HCV status identified and any true positives referred for treatment or close evaluation. The same would be true for those incarcerated or otherwise institutionalized. Testing could also be routine upon entry to college or vocational schools and is, I believe, already mandatory in the military service. While these measures would still not capture those who do not seek medical care, higher education, or military service, it would capture a large number of currently unidentified carriers and would be well worth the cost of testing.

Nonetheless, testing is only fully meaningful if it is the first measure on the path to curative therapy. Thus, the second major impediment to HCV eradication in the USA is access to care. Currently, many persons already identified as HCV carriers have not been treated because the cost of treatment is so high that is beyond the bounds of most individual health budgets and is not covered by many insurance companies. Many patients have thus far been treated in clinical trials and compassionate use protocols, but this is the minority, and some balance must be reached between industry, insurance companies, and the government to find a middle ground that assures reasonable, but not usurious, profits and wide access to treatment even among the most indigent and those whose habits have put them at risk. The treatment of intravenous drug users, for example, is a strong public heath measure because every carrier taken out of a needle-sharing population diminishes spread within that population, a community which is now the primary incubator of new HCV infections. Since new treatments are simple and with few side effects or drug interactions, they can be administered not only by hepatologists, gastroenterologists, and infectious disease specialists but also by general practitioners, nurses, and nonphysician support staff [51]. Hence, if drugs were available at affordable and reimbursable

cost, there would be no lack of medical personnel to administer and oversee treatment.

Thus, in the USA and most developed nations, the primary impediments to the potential for HCV eradication are the need to identify the large mass of silent carriers and then to triage them into treatment programs that are not limited by cost. This is a tall order, but not impossible if there is a collective will to do so. A universal HCV vaccine would be the long-term solution, but as with HIV, vaccine development has been hindered by the quasispecies nature of the virus and the failure of vaccines developed to date to induce broadly protective neutralizing antibodies or adequate T-cell responses. If such a vaccine were to be developed and proven safe and efficacious in clinical trials, it would be at least a decade before it was available for mass vaccination. Hence, the current strategy is to "test and treat," and both parts of that equation must be expanded dramatically.

The dilemma of HCV treatment is greatly compounded in developing nations where the routes of HCV spread are multifactorial, where access to care of any kind is limited, and where financial constraints to treatment are maximized. Thus, global eradication of HCV in the absence of vaccine will require strategies to change medical practice to eliminate the reuse of needles and multidose vials and syringes, cultural changes in practices of scarification, tattooing and ritual surgery, and the infusion of HCV therapeutics and financial resources from world health communities, resource-rich governments, private philanthropy, and industry. The PEPFAR program for HIV surveillance and treatment could serve as proven model.

So the end is in sight, though still far down the pike, but clearly we are at the beginning of the end, and it is the remarkable recent advances in HCV therapeutics that have made this vision possible.

Compliance with Ethical Standards

Ethical Statement: All patient studies described herein were performed under NIH IRB approved protocols with appropriate informed consent.

Chimpanzee studies were approved by the animal use committees of the Southwest Foundation for Biomedical Research, San Antonio Texas or the NIH, Intramural program.

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