

The Hepatitis C Virus: Your Part in its Downfall

Getting to know the Virus and its Host

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Abstract

The cloning of the genome of the Hepatitis C virus (HCV) in 1989 provided sensitive screening tools and specific diagnostic assays which helped health workers to limit viral spread and scientists to design effective anti-viral agents. The uncorrected intrinsic error rate of HCV's key replicating enzyme (RNA-dependent RNA polymerase) leads to antigenically different "quasispecies" which allow the virus to escape immune recognition by the host and leads to an ill-sustained clearance of virus by T lymphocytes. This article discusses the factors which may lead to progressive liver fibrosis and provides a theoretical framework for the selection of patients for viral eradication using pegylated preparations of recombinant interferon in conjunction with the oral antiviral ribavirin.

Keywords

Hepatitis C, quasispecies, genotypes, viraemia, cirrhosis, fibrosis, transaminase

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Introduction

The remarkable discovery of the causative agent of non A non B (NANB) transfusion related hepatitis in 1989 and the subsequent availability of sensitive serological screening tests, made scientists and health workers acutely aware of the pandemic proportions of Hepatitis C virus (HCV) infections. The *tour de force* of successions of discoveries and breakthroughs that followed in this field has been overwhelming. The problem of prevention and treatment of chronic HCV viraemia challenged different health workers and laboratory scientists in different ways. Health workers in many different specialities are called on to contribute their part in the prevention and treatment of this challenging disease. This article aims to overview the developments that took place in this field over the last decade in order to allow different health workers to generate their own perspective of a multidisciplinary approach to this global disease. An understanding of the biology of the virus, its genetic diversity, and its interaction with the host will allow more meaningful participation in global eradication and control of HCV.

Meet your enemy

Until 1989, the putative viral particle responsible for transfusion related non-A non-B (NANB) hepatitis was known to be a 50nm particle that possesses an envelope which could be inactivated by organic solvents. During this year Choo *et al*¹ at the Chiron Corporation (Chiron Corporation, Emerville, California, 94608, USA) prepared a random complimentary DNA library from RNA and DNA present in sera obtained from NANB patients. After cloning in bacteriophages, over a million restriction DNA fragments were painstakingly screened, against sera obtained from chimpanzees chronically infected with the NANB virus until a reactive clone (clone 5-1-1) was identified. The entire genome of HCV was then sequenced from clones that overlapped this single reactive clone.

The HCV genome of this enveloped RNA virus is now known to consist of a single strand of RNA of positive polarity consisting of an open reading frame of about 9000 nucleotides flanked at the 5' and 3' ends by highly conserved untranslated regions. The 5' end of the genome encodes for familiar structural components such as the core protein and the envelope while the 3' end encodes for a number of non-structural components which include the enzyme, RNA-dependent RNA-polymerase (RdRp) responsible for copying the whole genome² into an

intermediate strand of negative RNA from which daughter positive strains will be produced so as to infect neighbouring liver cells.

HCV is well known for its chronicity causing variable but often progressive liver damage which may proceed over as many as 30 years to liver cirrhosis. The key to the chronic nature of HCV viraemia lies in the properties of RdRp. The polymerase enzyme has an intrinsic error rate and regularly misincorporates wrong nucleotides when copying RNA. Unlike many other polymerases it has no proof-reading mechanisms.³ Many of the resultant (progeny) mutant virions fail to replicate but the few that survive possess a survival advantage because they are new to the immune system. In fact they constitute a new "quasispecies" which will continue to replicate until the immune system learns to recognize them, after which a new quasispecies will emerge, so that many patients are repeatedly challenged, over many years, by waves of viraemia involving new quasispecies. Patients who harbour large numbers of quasispecies are, not surprisingly, less likely to respond to treatment with immune-modifying agents such as interferon alpha.⁴

Another form of diversity is seen when we look at a cross section of strains of Hepatitis C viruses endemic in different parts of the world. Globally, one can distinguish at least 6 main "genotypes", each differing from the other by as many as 35% of the corresponding nucleotides. Each genotype is further subdivided into sub-types⁵ with approximately 20% variability in nucleotides. Genotypes are thought to have evolved from a common ancestral virus several hundred years ago. The predominance of certain genotypes in geographical areas (e.g. type 3 in India, and type 6 in Asia) probably results from population migrations. A broad spectrum of subtypes is usually found in a population which has harboured the virus for many centuries while a limited diversity of subtypes is usually seen in areas where the virus was introduced relatively recently (e.g. Australia). Genotypes 1a, 1b, 2a, 2b, and 3a are the commonest serotypes found in western Europe and North America. Preliminary data point to a possible preponderance of Genotype 1 in the Maltese islands. Genotype analysis is important because it predicts response to treatment and determines the dose and duration of modern antiviral regimens. Genotype analysis is now available in kits which perform in situ hybridisation using probes corresponding to the 5' non-coding region of the viral genome.

Know its whereabouts

The WHO 1999⁶ report contains prevalence data for seropositivity to HCV (i.e. significant titres of serum antibodies to HCV detectable by enzyme immunoassay) in 130 countries which range from 0.003% in Sweden to 18% in Egypt. In North America, where many of the scientific breakthroughs in HCV research were made, the reported prevalence rate is 1.8%. The high prevalence in Egypt is attributed to a large reservoir of HCV carriers resulting from mass treatment of schistosomiasis

with intramuscular tartar emetic that started in 1920 and was only replaced with oral treatment after 1982. Italy's reported prevalence is 0.5% but some rural areas report prevalences of up to 10% in the older age groups, attributed to the use of glass syringes and to unsterile dental practices in former times. A similar situation existed in Japan (overall prevalence 2.3%). Most countries base their reports on seroprevalence in blood donors and antenatal patients. This introduces selection bias and is only an approximate indication of the true prevalence in the population. In Malta 0.4% of potential blood donors were seropositive during the period 2000-2001.

Transmission of HCV viraemia is predominantly by the parenteral route. Most (50% - 80%) intravenous drug abusers (IVDA) who share needles or related paraphernalia seroconvert during the first year of IVDA and 100% are seropositive after 8 years. Compared to the Hepatitis B virus (HBV) sexual transmission to a single life time partner is low but rises with promiscuity, homosexual practice and after sex with a HIV-HCV coinfecting partner, all conditions in which undisclosed needle-sharing is likely to occur. The risk of sexual transmission of HCV is estimated to be 0.03% to 0.6% per year for partners in a monogamous stable relationship and 1% per year for those with multiple sexual partners. Stable partners may be infected with the same genotype that is prevalent in the area through non-sexual routes. Studies based on viral sequence analysis, rather than genotype, in HCV-concordant couples suggest that this is the case in as many as 50% of such couples.⁷

Vertical transmission to the new born has been reported to occur in approximately 5% of births but the risk is higher (5-36%) if the mother is HIV-HCV coinfecting⁸ or if the mother's serum HCV RNA levels exceed 10^6 copies per ml. Many infants born to viraemic mothers are seropositive because of passive transfer of IgG anti-HCV antibodies across the placenta. Persistence of HCV RNA in the infant's serum on two occasions 3-4 months apart after the infant is 2 months old and / or detection of antibodies after the age of 18 months are more reliable indicators of chronic infant viraemia.⁹ It is still not known whether such infants are less likely to clear viraemia than adults, as is the case with the HBV.

While as many as 8% of patients receiving multiple transfusions prior to 1989 seroconverted, the risk of chronic HCV viraemia after blood transfusion is now very low (0.001%/unit). Seroconversion during long-term haemodialysis occurs at about 0.15% per year. Needle-stick injury with hollow-bore needles is associated with a higher risk of seroinfection (0.8%) than other sharp injuries. A study, by the author of 54 patients referred to the liver clinic at St. Luke's Hospital for chronic HCV viraemia revealed IVDA in 34 patients, transfusion-related hepatitis in 4 patients, needle-stick injury in 1 patient and intramuscular injections with glass syringes in childhood in 4 patients as the probable source of infection.

Individuals with high-risk behaviour as well as patients with unexplained elevations in serum transaminases should therefore be screened for HCV. The use of alanine

aminotransferase (ALT) as a surrogate marker in blood donors did much to prevent the spread of HCV in the early days. However ALT is known to fluctuate and may be falsely negative in mild disease. By 1989, soon after the viral genome was cloned, a first generation enzyme immunoassay (EIA) with a sensitivity of 80% became available. The sensitivity has improved to 99% in currently available 3rd generation multiantigen assays. When used with a low cut-off threshold to screen for HCV in a low prevalence areas, as is likely in Malta, EIA gives too many false positive results and further confirmation with a recombinant immunoblot (RIBA) which visualizes peptide targets in the patient's serum or with a reverse transcriptase polymerase chain reaction (PCR) assay is required. PCR, though more expensive, is the gold standard required to diagnose HCV viraemia and to confirm its eradication after treatment because, unless samples are contaminated, it provides irrefutable evidence of circulating viral RNA. PCR should also be performed in immunocompromised patients who may have false negative EIA results. In-house PCRs have now been largely replaced by commercial PCR kits such as that used at St. Luke's Hospital which has a typical sensitivity of 50 viral copies per ml. Variations in PCR sensitivity between different brands, due to differences in the efficiency of reverse transcriptase or in the nucleotide probes, is now being minimised by standardization. Although copies per ml and IU per ml cannot be directly converted, 1000 copies per ml equals approximately 400 – 500 IU per ml. Quantitative PCR (wherein a known concentration of tag is amplified together with the DNA transcribed from the RNA in the sample) is less sensitive but is used to predict whether anti viral therapy should be continued after the first 12 weeks of treatment .

Get to know its ways inside the host

HCV circulates in plasma bound to a carrier protein (probable low density lipoprotein) until it binds to a receptor (probably CD 81 or LDL) on the hepatocyte membrane, enters the cell by endocytosis, and uncoats, shedding the viral envelopes in the cytosol. The capsid (core protein) is also lost before the viral RNA lands at the ribosomes where the polymerase RdRp translates the negative RNA strand (viral genome) into an intermediate positive strand which replicates in the cytoplasm producing progeny RNA, which after being fitted with a new capsid and envelope in the Golgi leaves the cell by exocytosis to infect neighboring hepatocytes.

Although HCV has been shown to be directly cytopathic to liver cells in high concentration, many hepatocytes are killed by activated CD8+ cytotoxic T lymphocytes (CTLs) through the induction of apoptosis (accelerated cell death) by the action of lymphokines such as TNF \pm and by the "drilling" of lethal pores in the hepatocyte membrane by pore-forming proteins called perforins. Unfortunately, except when continually boosted by large doses of administered recombinant interferon, the cell-mediated clearance of doomed virally-infected hepatocytes is only partially effective and is periodically overwhelmed by waves

of accelerated viraemia so that enough virus often survives to infect healthy neighboring hepatocytes. A humoral response is also mounted by activated B-lymphocytes but most of the relevant viral envelope antigens are highly variable and often escape recognition by neutralizing antibodies or experimental vaccines.

During the early phase of chronic viraemia, liver biopsies reveal a diffuse infiltrate of T and B lymphocytes. Over the next few years periportal hepatocytes die off and there is extension of hepatocyte necrosis across the, now jagged, portal limiting plate (interface hepatitis or piecemeal necrosis). More sinister is the appearance of fibrosis, initially limited to the periportal area but in certain patients bridging the portal areas and extending to the central vein of the hepatic lobule. The fibrous tissue may completely encircle regenerative nodules so that liver architecture is completely disrupted by cirrhosis.

Identify its potential victims

Until cirrhosis develops HCV viraemia is largely asymptomatic. From this point onwards morbidity, mortality and quality of life deteriorate. Typically 4% of cirrhotic patients decompensate every year so that within 10 years, more than 30% of patients will have suffered ascites, variceal bleeding or encephalopathy and 20% will have developed a hepatocellular carcinoma, although many of these tumors will not yet be apparent clinically. Survival drops from 90% at 5 years to 80% at 10 years after onset of liver cirrhosis. Liver biopsies stained with H&E and Masson's trichrome stains allow quantification of liver damage, using practical scoring systems such as the modified histology activity index (HAI) score for necroinflammation (previously known as the Knodell score) (grade 0-4)¹⁰ and the French METAVIR score for fibrosis (stage 0-4).¹¹ However it is not easy to predict whether, and how rapidly, a patient may progress to cirrhosis. Retrospective studies of the natural history of HCV viraemia are often limited by uncertainty about the assumed date of onset of asymptomatic viraemia. Prospective studies in high-risk groups incur selection bias as asymptomatic patients are more likely to default follow-up. Non-concurrent prospective studies following a common source outbreak such as the study by Kenny-Walsh *et al*¹² of 62,660 Irish women approximately 17 years after suspected exposure to contaminated antiglobulin may not be representative of HCV viraemia acquired in the community which may involve repeated infections with different viral strains during IVDA. In three large population-based studies of community-acquired HCV viraemia by Poynard *et al*¹³ analysis of 2335 liver biopsies predicted that fibrosis progresses at a median rate of 0.133 METAVIR units per year. It is now generally agreed that the patients age, age at onset of viraemia, male sex and alcohol consumption all predict more rapid progression of liver fibrosis. Immune suppression (e.g. by HIV co-infection) also accelerates fibrosis presumably by encouraging viral replication. Patients with normal serum ALT often have low fibrosis scores but liver biopsy is still necessary

as exceptions occur. Viral genotype was found not to predict fibrotic progression. Studies in referral centres such as the one by Tong *et al*¹⁴ showed that fibrosis progresses at an exponential rate. Patients who may take 20 years to progress to moderate fibrosis often progress to cirrhosis in a much shorter interval.

Conclusions

Health workers need to be made aware of the spectrum of interactions between the virus and its host both before and during attempts at eradication of chronic viraemia. Such knowledge will lead to improved referral and selection for treatment. This will limit expense and lead to more frequent sustained viral clearance. A basic understanding of the mechanisms by which the virus may evade complete elimination by antiviral regimens helps to tailor individual therapy using the right combination of dose and duration. Many of the questions asked by patients referred for antiviral therapy cannot be answered without a basic awareness of strategies used by the virus to avoid immune clearance.

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