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The Irish paradigm on the natural progression of hepatitis C virus infection: An investigation in a homogeneous patient population infected with HCV 1b (Review)

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Abstract. The aetiological agent of chronic hepatitis C is the hepatitis C virus. The hepatitis C virus is spread by parenteral transmission of body fluids, primarily blood or blood products. In 1989, after more than a decade of research, HCV was isolated and characterised. The hepatitis C viral genome is a positive-sense, single-stranded RNA molecule approximately 9.4 kb in length, which encodes a polyprotein of about 3100 amino acids. There are 6 main genotypes of HCV, each further stratified by subtype. In 1994, a cohort of women was identified in Ireland as having been iatrogenically exposed to the hepatitis C virus. The women were all young and exposed as a consequence of the receipt of HCV 1b contaminated anti-D immunoglobulin. The source of the infection was identified as an acutely infected female. As part of a voluntary serological screening programme involving 62,667 people, 704 individuals were identified as seropositive for exposure to the hepatitis C virus; 55.4% were found to be positive for the viral genome 17 years after exposure. Of these women 98% had evidence of inflammation, but suprisingly, a remarkable 49% showed no evidence of fibrosis. Clinico-pathology and virological analysis has identified associations between viral load and the histological activity index for inflammation, and, between inflammation and levels of the liver enzyme alanine aminotransferase. Infection at a younger age appears to protect individuals from progression to advanced liver disease. Molecular analyses of host immunogenetic elements shows that particular class II human leukocyte associated antigen alleles are associated with clearance of the hepatitis C virus. Additional class II alleles have been identified that are associated with stable viraemia over an extended period of patient follow-up. Although, investigation of large untreated homogeneous cohorts is likely to become more difficult, as the efficacy of anti-viral therapy improves, further investigation of host and viral factors that influence disease progression will help provide an evidence based approach were realistic expectations regarding patient prognosis can be ascertained.

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1. Epidemiology and natural history of hepatitis C virus infection

Chronic hepatitis C is a ubiquitous disease, affecting approximately 170 million individuals worldwide (1). The global prevalence of the hepatitis C virus (HCV) ranges from less than 0.5% (Scandinavia, USA, Western Europe) to greater than 44% (Northwest Egypt and southern Cameroon) (2). The HCV is spread by parenteral transmission of body fluids, primarily blood or blood products. In 1989, after more than a decade of research, HCV was isolated and characterised (3). The hepatitis C viral genome is a positive-sense, single-stranded RNA molecule approximately 9.4 kb in length, which encodes a polyprotein of about 3100 amino acids (4). This polyprotein is cleaved into functional proteins by cellular and viral proteases. There are 6 main genotypes of HCV, each further stratified by subtype (5). Hepatitis C virus exists as a heterogeneous mixture of closely related viruses called quasispecies. Substantial evidence indicates that HCV genotype is clinically important with respect to efficacy of interferon therapy, the likelihood of response to antiviral therapy appears to be associated with the degree of quasispecies diversity (6-9). Additionally, mutations in the viral population likely contribute to the emergence of resistance during anti-viral therapy.

Current estimates of medical and work-loss costs of HCV related acute and chronic liver diseases are greater than $600 million per annum in USA (10). Ireland, like many other countries is no stranger to the HCV, has been effected by the considerable socio-economic cost of the HCV. The Hepatitis

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Compensation Tribunal set-up in response to the iatrogenic infection of Irish citizens with HCV has from 1996 to 2000 costs of approximately 380 million Euro (11). HCV associated end-stage liver disease is the most frequent indication for liver transplant among adults (10). Since, most HCV infected individuals are relatively young (18-40 years) the number of deaths attributable to HCV related disease is likely to increase substantially in the next two decades.

Serological evidence of exposure to the HCV can now be identified by both enzyme linked immunosorbent and recombinant immunoblot assays (12). Detection of viral nucleic acid is possible using an array of nucleic acid technologies (12). Acute hepatitis C is often mild and asymptomatic. Chronic hepatitis C, is an indolent disease characterised by hepatic inflammation and hepatocellular necrosis, may progress to cirrhosis and/or hepatocellular carcinoma (1,13,14). Our understanding of why only 45-20% of individuals resolve a primary infection is limited, but recent findings of human leukocyte associated antigen correlations with viral clearance may offer insights into how potential vaccines can be developed (15-25).

Many studies have attempted to define correlations between the indices of disease activity in an endeavour to define the natural progression of chronic hepatitis C (26-32). However, there is considerable heterogeneity with regard to the finding of associations between biochemical, histological, and virological markers of disease. This conflict is likely to be due, in large part, to the heterogeneity of patient groups with respect to the variation in disease duration, differences between size and 'relative fitness' of individual viral inocula, mixed genotype study groups, mixed gender study groups, co-existence of hepatotropic infections and immunomodulatory viruses such as the human immunodeficiency virus (HIV). Defined homogeneous cohorts offer the best opportunity to clarify the true natural progression of hepatitis C infection. Globally, there are two relatively homogeneous cohorts of individuals infected with HCV 1b from contaminated anti-D immunoglobulin during the late 1970s (15,16). An additional cohort where long-term concurrent prospective evaluation of disease activity is under investigation is the Veterans Administration (VA) study of transfusion-associated hepatitis conducted by Seeff *et al* (33). The value of these study groups is that the time of infection has been established, the anti-D cohorts are ethnically homogeneous, in particular the anti-D cohorts are all infected with the same genotype/subtype of the HCV, infection was from a single donor, and a high percentage have undergone and are likely to undergo continuous long-term prospective follow-up.

2. The Irish paradigm on the natural progression of hepatitis C virus infection

An investigation in a homogeneous patient population infected with HCV 1b
Background to iatrogenic infection of Irish women in Ireland with HCV 1b from contaminated anti-D immunoglobulin. In 1993 a group of Irish individuals were identified as seropositive for exposure to the hepatitis C virus during an investigation of the prevalence of hepatitis C seropositivity in the Irish blood donor population. In total, 29 individuals out of a total of 100,000 donors screened, were seropositive (34). Fifteen were female and 14 were male. Of the 15 female individuals 13 were found to be Rhesus negative. The finding of nearly 87% Rhesus negative individuals in this small group of individuals was inversely proportional to the observed frequency for this marker in the Irish population. This observation prompted the initiation of a lookback study to identify any infectious or potentially infectious commonality among these 13 women. Twelve of the 13 individuals were found on lookback to have been in receipt of anti-D immunoglobulin during 1977-78. Subsequent molecular screening for viral nucleic acid revealed that batches of anti-D immunoglobulin used in Ireland during the 18 month period from May 1977 to November 1978 were found to be contaminated with HCV genotype 1, subtype b (16,35,36). Molecular evidence for transmission via HCV 1b contaminated anti-D immunoglobulin (HAD-1b) was also provided by comparison of sequences from 100 individuals for part of the NS5 region of the viral genome and virus isolated from implicated batches of anti-D immunoglobulin 17 years after the exposure event (37). The iatrogenic infection of Rhesus negative women in May 1977 to November 1978 presented the scientific and medical communities in Ireland with an globally unique opportunity to study, the natural history of hepatitis C infection, and, the viral and host factors that affect the rate of disease progression.

Further investigation discovered that the sole source of the infectious plasma used to harvest the anti-D immunoglobulin was documented to be from a female individual who experienced a jaundice episode after transfusion, 4th November 1976 (34,36). In an attempt to identify all possible recipients of potentially infectious anti-D immunoglobulin an anti-HCV serological screening programme was instituted in the Republic of Ireland (36). In total, 62,667 individuals were screened in a voluntary nation-wide screening programme (16). Seven hundred and four individuals were identified as having evidence of past exposure to HCV. Of these individuals 55.4% were subsequently identified as being positive for the HCV genome, all were confirmed as HCV 1b.

Histological disease progression 17 years post-infection. Extensive evaluation of 376 (96%) of those who were positive for the viral genome indicated that approximately 33% had at least one other risk factor for hepatitis C, in addition, to exposure to HCV 1b contaminated anti-D immunoglobulin (16,38). The most common other risk factor was blood transfusion at 17%. The median ALT level was 42 IU/ml. Histologic appraisal of 93% (n=363) of those who were PCR positive for HCV indicated that a remarkable 49% had no evidence of fibrosis and only 2% had evidence of probable or definite cirrhosis. In the absence of competing aetiologies for liver disease, hepatitis C infection appears to progress slowly in this study group infected with HCV 1b. The relatively benign outcome of this infection, in this cohort, may reflect the small size of viral inoculum, the relative young age at infection, the observation that disease progression is slower in females, or, that further concurrent prospective follow-up is needed to determine the natural progression of what is an infection of several decades. Interestingly, of the 2% (n=7) with evidence of cirrhosis in the Irish anti-D cohort, two individuals reported excessive alcohol intake. The significance of the

findings reported by Kenny-Walsh are that they for the first time defined what is likely to be the natural progression of chronic hepatitis C, in the absence of competing aetiologies for liver disease and in a cohort where alcohol consumption is low. Investigation of a German cohort of women infected through HCV 1b contaminated anti-D immunoglobulin reported similar histological findings to Kenny-Walsh (16).

Clinicopathology and virology of HAD-1b infected individuals. Several investigators have defined the clinicopathological and virological features of chronic hepatitis C infection. However, there has been considerable heterogeneity with regard to the ascribed associations. The dichotomy between individual investigations is likely to be the result of variation in viral genotype, variation in disease duration, combination of associated risk factors for more rapidly progressing liver disease such as alcohol, hepatitis B virus co-infection, HIV, variance in the age at infection and the probably risk factor of multiple exposures. The strength of the Irish anti-D cohort is the ability to control for variables that impact on the natural history of HCV infection. In a study population of 77 PCR positive individuals from the anti-D cohort, we identified an association between i) HCV RNA titres and the degree of inflammation, and ii) the degree of inflammation and serum ALT levels, 17 years post infection (32). Correlative analysis between the indices of disease activity by its nature has to assume that the 'snap-shot' view of any disease is representative of the overall progression. The observed associations between clinicopathological and virological indices of disease represent a window into the natural history of this infection. These correlations may not withstand prospective evaluation as there is currently no evidence that disease progression in chronic hepatitis C is linear. Mechanistic explanations for many of the observed correlations are currently unclear. The mechanisms of HCV-induced liver damage are not clearly defined. However, hepatic injury is likely to be, in part, immune mediated because HCV is not obviously cytopathic. The evidence that HCV-associated hepatic injury is immune mediated is primarily indirect: i) treatment with corticosteroids and other immunosuppressive agents can reduce ALT levels even though viral load increases (39,40); ii) HCV-infected liver transplant patients, when immunosuppressed, have very high viral titres but can remain asymptomatic (40); iii) activated CD8+ T cells can migrate from the periphery to the liver and initiate tissue damage (41) and iv) viral replication can occur in the absence of evidence of liver cell damage (42). The immunologically mediated necroinflammatory injury see in hepatitis C infection is some what akin to the situation observed in chronic hepatitis B infection (43). Suggestive evidence of a role for HCV core and NS3 protein in development of hepatocellular carcinoma may indicate that perhaps the gene products of HCV have oncogenic potential (44-47).

Host-virus interaction. Host factors that influence disease progression include age at exposure, excessive alcohol consumption, and the presence of competing aetiologies for liver disease. In addition, host-dependent genetic factors, (specifically, HLA class II), have been shown to be related to viral clearance in several studies (20-23,25,48-51).

We undertook an investigation of the HLA class II elements associated with persistence or clearance of HAD-1b in a study population of 156 from the Munster region of Ireland. All 156 individuals were confirmed RIBA positive, with 46% of these individuals (n=72) positive for the HCV by RT-PCR. HLA DRB1 and DQB1 status was molecularly defined by high resolution reverse line probe hybridisation methodologies (22). Several factors were identified as having an association with clearance of HAD-1b, specifically, DRB1*01 (excluding the DRB1*0103) and the extended haplotype of DRB1*0701/ DQB1*0501. Two other independent studies on persistence and clearance in HAD-1b exposed individuals have reported the association of DRB1*01 with clearance of HCV (21,23). Thio *et al* also reported an association of HLA DRB1*01 alleles with clearance of HCV (18). In our particular study group, no association between individual alleles of the DQB1 locus and persistence or clearance was found. However, McKiernan *et al* identified DQB1*0301 as having an association between exposure to HAD-1b and resolving infection (21). This finding is in agreement with that reported by others (20,49,52). Globally, the variance of association between different study populations is likely to reflect several important factors i) the allelic possession frequency between ethnically different populations (18,53), ii) differences between populations or skewing of results caused by comparisons between infected individuals and unexposed controls, as opposed to individuals who were exposed to HCV and either cleared the infecting virus or developed chronic persistent infection (20), and iii) mixed genotype study populations with variation in disease duration (18,49).

An examination of possible host factors that may influence the dynamics of hepatitis C viraemia revealed that particular HLA class II alleles, or genes in linkage disequilibrium with these alleles, were associated with either stability of viraemia or dynamic change in viral load during the study period for this study group (54). High resolution molecular analysis of the HLA class II DRB1 and DQB1 loci revealed that changes in viraemia were associated with DRB1*15/DQB1*0602 haplotype. It was found that possession of DRB1*0701/ DQB1*0201 haplotype was associated with relatively stable viral load over an extended period of patient follow-up. The mean fold increase per annum during the study period was approximately 1.3-fold increase during the study period for the latter haplotype. Those individuals who were in possession of DRB1*15/DQB1*0602 were found to have a mean fold increase in viral load per annum of 6.1. The finding of a dynamically changing viral load for those individuals in possession of the DRB1*15/DQB1*0602 haplotype may reveal a window of transformation in the natural history of this infection that superseded a period where the dynamic fitness of the viral quasispecies was set a presumptive zero. The significance of these data is that it shows that host genomic factors can influence the magnitude of hepatitis C viraemia. Definition of those viral and host factors associated with stable or changing viral load may offer opportunities for the manipulation of viraemia to create a clinically advantageous arena for improving the efficacy of current and future anti-viral therapies.

Person-to-person variability in blood levels of HCV RNA is likely to result from a dynamic equilibrium between the

host factors that influence the rate of i) viral clearance, ii) elimination of infected cells and iii) the viral factors that influence the rate of infection of naive hepatocytes and iv) factors that influence the efficiency of production of infection competent daughter virions. In a study aimed at isolating the determinants of viral load Thomas *et al* examined viral loads in 969 persons who acquired HCV infection in the context of injection drug use (55). However, greater than 90% of the person-to-person HCV RNA level variability could not explained by the sociodemographic factors of HCV and HIV co-infection, HCV and non-HIV co-infected individuals, ongoing hepatitis B infection, age and the absence of needle sharing. A few studies have tried to elucidate possible hepatitis C viral genomic sequences associated with fluctuations in hepatitis C viral load. Terazawa *et al*, suggested that mutations in the interferon sensitivity determining region (ISDR) are associated with changes in viral load over a relatively short period of time (56). These findings are suggestive of a biologically relevant phenotype associated with changes in viral load. However, the study group was relatively small, reducing the significance of the inference. Another Japanese study by Hashimoto *et al*, looked at the associative relationship between the inferred amino acid sequence of the hypervariable region 1 (HVR I) and the ISDR and fluctuations in viral load, but found no significant association over a 1-2 year period of patient follow-up (57). This dichotomy between these two Japanese studies, is likely due to heterogeneity of patient population, sequence heterogeneity of the infecting viruses, although both studies were of individuals infected with HCV 1b, and variance in the duration of disease.

Interperson variability in viraemia was investigated in the HAD-1b group of 47 patients (58). None of the patients included in the study had received anti-viral therapy. A statistically significant correlation between age at exposure to HAD-1b and baseline viraemia at initiation of the study was observed. Those infected at a younger age tended to have much lower viraemia than those of more advanced years. If low viral load is associated with lower indices of disease activity this may explain, in part, why infection at a younger age 'protects' from rapidly advancing disease. The rate of change of viraemia during the study period was negatively correlated with the baseline viral load {rate of change was defined as the change between the first and last log10 HCV levels divided by the follow-up time between baseline and last follow up: rate of change of viral load was calculated using; rate of change = log10 [HCV (Tlast) - log10 HCV (Tfirst)]/ [Tlast - Tfirst]}. Thus, individuals who had a high viral load at baseline were found to have a slower rate of change of viral load per annum, than, those who had low baseline viraemia. The competitive exclusion principle may help explain, in part, these observations. In essence, the viral load set point at any given time is the result of the dynamic equilibrium between viral production and clearance (59). Stability of viral load between two time points is likely to reflect a situation where the fitness of the viral swarm fulfils the Red Queen Hypothesis. Displacement events that give rise to changes in viral fitness can perturb this dynamic equilibrium engaging the competitive exclusion principle (60-62). Thus, if a displacement event gave rise to an increase in 'fitness' of the viral swarm then the downstream effect of this is likely to be an increase in production of daughter virions and/or an increase in viral infectivity. The efficacy of the immune response to these 'new' antigenically different virions would of course affect the overall outcome of this displacement event. A new equilibrium is then likely to be established and the relative fitness of the population stabilises and a new set point where the balance between the advantages and deleterious mutations is effectively set at zero. Of course, the phenomenon of molecular memory in the viral quasispecies could be a disadvantageous event as immune memory previously established could open new avenues for combating this viral infection (63). As is evident from the few studies that have investigated the natural variation in hepatitis C viral load additional host and viral factors, yet to be defined, are likely to influence this dynamic.

3. Conclusions and remarks

The natural progression of chronic hepatitis C infection is not fully ascertained. There is conflicting data regarding the percentage of individuals who are infected who will ultimately have an acute resolving infection. Initial data suggest that this could be as low as 15%, but more recent Irish cohort studies suggest that this may be as high as 50%. There is a hetero-geneity of opinion regarding the long-term outcome of this chronic infection ranging from the belief that progression to cirrhosis and/or hepatocellular carcinoma is the normal course, to the belief that progression is indolent, resulting in mild to moderate disease in most individuals where development of cirrhosis is uncommon during the first two decades of the infection and where external factors (hepatitis B co-infection, HIV, alcohol) will result in a more rapidly progressing disease. Strategies used to determine the natural history of hepatitis C includes the retrospective, prospective and the combination study of retrospective/prospective cohort studies. Of course referral bias to specialist centres is an issue which may skew our understanding of the progression of this disease. Progression of liver disease in chronic hepatitis C is effected by both host (duration of disease, age at infection, gender, co-existence of hepatotrophic viral diseases, co-infection with immuno-modulatory viruses, immunogenetic background) and viral factors (genotype, viral load, quasispecies diversity, patho-genicity of viral isolate).

The investigation of hepatitis C viral infection in Ireland advances the following paradigm. That hepatitis C viral infection with genotype 1b, is likely to be resolved in nearly 50% of individuals who have evidence of past exposure. Histological progression nearly 20 years post-infection, shows that most individuals will have inflammation of the liver parenchyma and that nearly half of those with chronic infection show no evidence of fibrosis/cirrhosis. Of those with fibrosis only a very small percentage will progress to develop probable or definite cirrhosis. Abstinence from alcohol or minimal intake reduces the progression to cirrhosis. Infection at a younger age protects from progression to advanced liver disease is now evident from the investigation of the anti-D cohort. The set point of viraemia also appears to be influenced by the age at exposure. Those individuals who are exposed in early adulthood showed lower baseline viral load 17-19 years post-exposure of HCV 1b. Host immunogenetic factors

associated with clearance of HCV have been described. Host immunogenetic factors seem to be involved in protection from dynamic changes in viraemia. However, not all the observed changed in viral load over an extend period of patient follow-up could be accounted for by host HLA profile.

More research into the natural history of hepatitis C is required. Defined cohorts who are prospectively evaluated offer the best opportunity to define the natural progression of this chronic liver disease. Although, investigation of large untreated cohorts is likely to become more difficult as the efficacy of anti-viral therapy improves it will be essential to define those factors that will predict long-term outcome. However, most importantly, a greater understanding of the natural progression of hepatitis C and the factors that impinge on progression will provide for an 'evidence based' medical approach whereby realistic expectations regarding outcome can be achieved.

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