

Discussion

Examination of the value of treatment of decompensated viral hepatitis patients by intentionally coinfecting them with an apathogenic IBDV and using the lessons learnt to seriously consider treating patients infected with HIV using the apathogenic hepatitis G virus

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Abstract

Hepatitis virus infection persistent worldwide (~600 m people) results in chronic hepatitis progressing to hepatocellular carcinoma (HCC) in many (~1 m deaths/year). The review examines the usefulness of treating chronic viral hepatitis, including decompensated patients, by intentional coinfection with an attenuated infectious bursal disease virus (IBDV; apathogenic in man, stable at pH 2, orally administered). Learning lessons from the IBDV studies, the case is made to treat human immunodeficiency virus (HIV) infected patients (worldwide prevalence ~50 m people) by coinfecting with apathogenic hepatitis G virus (GBV-C). These ideas are reinforced by (i) eight out of ten studies reporting a beneficial effect of GBV-C viremia on HIV-related mortality or response to therapy and (ii) the recent reports of improved or delayed survival of HIV patients, naturally coinfecting with an apathogenic virus.

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1. Successful intentional coinfections by apathogenic avian bursal disease virus in hepatitis

Viral hepatitis is a serious global public health problem and also an economic burden. Worldwide, nearly 600 million people have persistent hepatitis virus infection, a constant source of chronic hepatitis [1,2]. Treating chronic viral hepatitis is still disappointing and realistically, the prospects

of an HCV vaccine remain remote. Not unexpectedly, hepatocellular carcinoma (HCC) accounts for almost 1 million deaths per year [3–9]. The idea that ‘desperate situations need desperate measures when no treatment available’, promising laboratory studies led to courageous novel treatments that benefited patients. Preliminary evidence in one to three patients then led to several remarkably useful trials extended world wide later. The examples are (i) cord blood transplantation therapy (Gluckman et al., 1989, Hôpital St. Louis, Paris [10]) that is now a treatment of choice; (ii) based on in vitro observations in scrapie-infected neuroblastoma cells, acridine and phenothiazine derivatives were proposed as a treatment for prion disease, including Creutzfeldt-Jakob disease (CJD), and a new variant CJD ([11]) and ‘slowed down’ disease by compassionate treatment with quinacrine and chlorpromazine in one or two patients has led to

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international trials; and (iii) cancer vaccine usefulness in just three patients (Baylor trials—JNCI, 2004 Feb 18, p. 326 [12]) forms the basis to extend trials.

The safety and efficacy of infectious bursal disease virus (IBDV) coinfection therapy were reported in 42 acute hepatitis patients (HBV and HCV) in a phase II clinical trial [13]. The encouraging evidence was that progression to chronic infection was marginally better in IBDV-treated patients than in the controls. Serendipitously, the highly significant and quite unexpected additional observation was that IBDV therapy was also effective in three decompensated chronic hepatitis patients (two HBV, one HCV), who went into long-lasting remission or were stabilized with significant clinical improvement [14].

Recently, we reported [15] on the fourth HCV patient, who had become resistant to conventional interferon, ribavirin, and thymosin treatment, developed decompensated chronic viral hepatitis, and received disability status. IBDV therapy improved alanine aminotransferase (ALT), aspartate aminotransferase (AST), and viral RNA levels. Importantly, during the treatment of patients it emerged that to ensure an “artificial viremia” by IBDV (not known to infect humans naturally), the viral preparation needed to be given in large doses and continuously over a long period.

Evidence for any inhibitory effect of the avian infection on the hepatitis B or C viral replication is likely to emerge when appropriate studies, first in vitro with model systems, will have been completed and extended to in vivo systems.

In view of the above mentioned trials, the success of the infectious bursal disease virus coinfection therapy in four well documented cases of chronic decompensated hepatitis patients [14,15], (and the demonstration of the efficacy and safety of IBDV coinfection therapy in 42 acute HBV and HCV patients [13]), “deserves cautious trials.” (Three of these patients are well, in good spirits and capable of light work without IBDV medication [in July, 2004], whereas one patient died of liver cancer in 2003).¹

The main thrust and objective of this paper is to stimulate at least a serious debate about cautious trials of “IBDV to treat chronic hepatitis and extend to human immunodeficiency virus (HIV)/AIDS” [16], ensuring no influence of any competing commercially supported treatment developments. Furthermore, we also propose well established experimental cell systems (e.g. RNA interference

(RNAi)) to test the validity and usefulness of coinfection strategies for the treatment and control of hepatitis and HIV-AIDS.

2. Persistent hepatitis virus infection remains a serious global public health problem despite changing epidemiology of hepatitis viruses

The term hepatitis virus is reserved for those viruses that are predominantly hepatotropic. The hepatitis viruses can be broadly divided into those transmitted via the fecal-oral route and by body fluids, such as blood and blood products. Hepatitis A (*picornaviridae*), hepatitis B (*hepadnaviridae*) and hepatitis C (*flaviviridae*) represent the major public health problems. The epidemiology of hepatitis A virus (HAV) and hepatitis B virus (HBV) is changing in response to vaccination. Chronic hepatitis B in some regions is now predominantly of the so-called precore mutant type where high levels of HBV replication persist in the presence of anti-hepatitis B virus (HBe) antibodies [17]. When hepatitis B, C persist in a chronic carrier state, they serve as a reservoir for infection and give rise to chronic hepatitis and cirrhosis that usually though not invariably progress to hepatocellular carcinoma [18].

Despite the availability of a long established, safe and effective prophylactic vaccine, approximately 6% of the world's population (~400 m people), suffers from chronic hepatitis B viral disease that has remained a tenacious scourge, ranking ninth globally among all causes of mortality (up to 1 million deaths annually) [19]. As by 2000 only 116 of 215 countries, representing 31% of the global birth cohort, had adopted the integration of hepatitis B vaccine into existing childhood vaccination schedules, elimination of HBV transmission will not have occurred for decades [20]. Only a minority of infected adult cases, whereas 90% of children under 1 year of age, develop chronic hepatitis [21]. The clinical spectrum of chronic liver injury ranges from mild inflammation to the end stage liver cirrhosis and HBV infection is responsible for ~70% of HCC cases globally [22].

Chronic HCV infection is the cause of an emerging global pandemic of chronic liver disease; it has an estimated worldwide prevalence of 170 million cases. The majority of infected individuals are qualified for therapy [21,23–25]. The phenomenon of quasi species evolution and other viral factors have been proposed to explain the immune evasion by hepatitis C virus (HCV) [26]. The evolution of HCV genotypes in women (infected by HCV contaminated anti-D globulin) and in chimpanzees suggested a role of the hyper variable region of E2 in HCV immunity. As immunity to the initiating virus strain develops, quasi species rapidly replace the predominant subtype [27]. The majority of HCV patients develop chronic hepatitis (often mild and asymptomatic), which may be progressive, evolving to significant liver disease (cirrhosis or hepatocellular carcinoma) in about 20% of cases after decades [21,28,29].

¹ It is important to recall that before the IBDV treatment, conventional therapy failed to stabilize the patients' conditions. One of the patients (a 67-year-old female with a HCV infection) was confined to bed, unable to support herself due to a severe malaise. In the second patient (57-year-old female with HBV infection), a portal hypertension developed, suggesting a very poor prognosis. In the third patient (a 24-year-old female with HBV infection) progressive jaundice, generalized oedema and hepatic encephalopathy occurred and a request for liver transplantation was refused because of the high-level viremia. The fourth decompensated chronic HCV patient had a 20-year-history of the disease. Conventional interferon, ribavirin and thymosin treatment was unsuccessful. He received disabled status. IBDV therapy proved effective, enabling him to work and enjoy life [14,15].

HCV is frequently associated with type II mixed cryoglobulinemia, a benign B lymphocyte proliferative disorder, which sometimes evolves to overt B-cell lymphoma. The HCV envelope protein E2 binds human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes. One consequence of this tropism is the activation of B lymphocyte clones with the consequent production of autoantibodies and cryoglobulins. The secondary event is the formation of circulating immune complexes which, having precipitated at an intravascular level, may cause part of the extrahepatic manifestations associated with these infections [30–32].

3. Treatment of chronic viral hepatitis still remains disappointing: conventional combination treatment despite interferons has limitations

Currently, three treatment options are available for chronic HBV infection, including monotherapies of subcutaneous interferon, oral nucleoside lamivudine and oral nucleotide adefovir dipivoxil. Unfortunately, these agents have not effectively and frequently been able to attain a 'cure' or complete eradication of the virus. Furthermore, these are ineffective if given when there is no ongoing hepatitis (i.e., normal ALT level) [33–35]. The onset of rapid resistance to lamivudine led to the development of the oral adefovir dipivoxil, which is effective and generally well tolerated in HBeAg-positive and HBeAg-negative patients chronically infected with wild-type or lamivudine-resistant HBV. Few resistant HBV mutants have emerged to date [36,37]. Interferon and lamivudine are rarely effective on HBeAg-negative patients [38]. In the Mediterranean basin, 30–80% of patients are HBeAg-negative and more than 80% of such patients do not respond to the current approved therapies. The combination of IFN-alpha2b and thymosin-alpha1 is better tolerated and more likely to induce a sustained response in HBeAg-negative chronic hepatitis B patients when compared to other currently available therapies [39].

The current standard combination of interferon-based therapies and ribavirin is effective in only 50% of chronic HCV patients. The overall impact of antiviral therapy in altering the natural course of HCV infection still remains uncertain. This is also partly because therapeutic trials involve narrow selection criteria that would exclude the majority of hepatitis C patients in the community. The ideal restricting conditions of clinical trials may not be generally applicable to the average practice setting [40,41]. In addition, the combination therapy is expensive, requires lengthy periods of administration, and is associated with significant side effects. Furthermore, no effective preventive measure, such as vaccination, is currently available [42]. Compared with conventional interferon alpha, peginterferon alpha-2a (40KD) that has improved pharmacokinetics, provides sustained therapeutic plasma levels, and can be administered once weekly. Peginterferon alpha-2a and ribavirin for 48

weeks produced significantly higher sustained responses than three times weekly interferon alpha-2b and ribavirin in patients with chronic hepatitis C [43]. Patients with genotype 1 infection have a 42–51% likelihood of response to 48 weeks of therapy. Those with genotypes 2 or 3 infection will respond to 24 weeks of therapy in 78–82% of cases [44]. Pegylated IFNs with ribavirin are the standard of care for treating patients with chronic HCV who have not been treated previously [45]. Unlike hepatitis B, there is still no effective treatment in preventing recurrent hepatitis C after liver transplantation [46].

It is also a serious problem that in patients infected with chronic viral hepatitis and treated with IFN-alpha during 1 year, a great incidence of depression and anxiety was demonstrated not only during IFN-alpha therapy but also even after the treatment was discontinued [47]. IFN-induced depression occurs more frequently in HCV than HBV patients and in women than men [48].

In this context, it is important to note that the administration of exogenous IFN in mice resulted in opiate-like side effects. This was probably due to the IFN-alpha molecule binding to opiate receptors and the associated low molecular weight endorphin-like moieties synthesized by lymphocytes being released and modifying function [49]. HuIFN-alpha (but not HuIFN-beta or HuIFN-gamma) is known to bind to opiate receptors *in vitro*, resulting in analgesia, catalepsy and immobilisation similar to beta-endorphin and morphine. The effects are reversed immediately or prevented by the potent opiate antagonist naloxone (a chemical congener of the agonist morphine), suggesting the opioid nature of the receptors. As opioid receptors are present also on human blood lymphocytes [50,51] (for lead refs. see [52]), the interferon related CNS side effects are not surprising.

Depression and general morbidity associated with IFN and lack of response require great attention in the sensitive management of patients. This is because of the real risk of feelings of hopelessness, depression, psychotic episodes and attempts at suicide [53,54]. Psychiatric side effects are known to lead to non-compliance, unfortunate and frustrating relapses in recovering alcoholics and drug addicts.

IFN therapy may also provoke autoimmune thyroid disease in HCV-infected patients, which can consist of autoimmune primary hypothyroidism, Graves' hyperthyroidism, and destructive thyroiditis, with hypothyroidism being the most common side effect [55,56]. The advent of PEG-IFNs has increased the severity of the hematological adverse effects [57].

Chronic hepatitis C is fast becoming the leading indication for liver transplantation. Liver transplantation is a therapeutic option for some but graft infection is universal and often complicated by progressive liver fibrosis. A vaccine remains a remote prospect so that prevention is crucial [21,23].

Clearly, better therapeutics and treatment strategies are needed.

4. Alternating viral dominance in dual infections may be exploited to treat persistent infections

Infectious agents and host defences have co-evolved to reach balanced states where virus and host survive [58]. DNA viruses that form persistent infections are thought to be the most likely candidates for phylogenetic congruence. Nevertheless, phylogenetic reconciliation analysis demonstrated that RNA viruses are also able to form stable associations with their hosts over evolutionary time scales and that the details of such associations are consistent with persistent infection being a necessary but not sufficient precondition [59]. Avian influenza viruses for example exhibit relative evolutionary stasis in their avian hosts [60], or simian immunodeficiency virus (SIV) seems to be non-pathogenic in the vast majority of natural hosts in spite of high levels of viral replication [61]. Other persistent viral infections, like human immunodeficiency virus, HBV, HCV, and others have not yet reached such an optimal balance.

Unfortunately, drug therapies against such persistent human infections fail to consistently eradicate the infection from the host, and vaccine-mediated protection against such viruses is also very difficult to achieve. For example, the herpes simplex viruses (HSV) cause lifelong persistent infections with numerous disease manifestations. Genital herpes infections are widespread in populations throughout the world and a vaccine to protect against or subdue established genital herpes infections has been under development for decades without success [62]. To tackle persistent infections new approaches are required. One of these could be to exploit the alternative dominance in viral replication.

Isolated case reports demonstrated that chronic hepatitis induced by a B virus resolved during an intermittent infection with an acute type hepatitis A [63]. Concurrent acute infection with hepatitis C virus inhibits acute hepatitis B virus infection and onset of hepatitis B may reduce the severity of hepatitis C virus infection but not frequency of chronicity [64]. The core protein of hepatitis C virus can suppress gene expression and replication of hepatitis B virus in a human hepatoma cell line (HuH-7) [65]. Dual or triple hepatitis virus infections are associated with viral interference, in particular, HCV exerts a suppressive effect on HBV and HDV and may enhance seroclearance of HBV antigens [66–71]. HBV infection seemed to suppress HCV replication even in HBsAg negative patients with dual infection [72–74]. Hung et al. [75] reported recently the case of a 66-year-old woman with acute HBV superinfection, which occurred during follow-up of chronic HCV infection. The patient developed hepatic decompensation at the acute stage, which was followed by a virologic remission with undetectable HBV DNA and HCV RNA during nine months of follow-up. It was indicated that these viruses show alternating dominance in replication in most of the patients, who have dual infection with HBV and HCV, probably due to interference of the viruses [76–78]. Recently, sequential HBV DNA levels in stored serum samples obtained from nine men with chronic HBV, who acquired

HIV infection, were evaluated [79]. Quite unexpectedly, five men had a mean decrease of 6.29 log₁₀ copies/mL in the HBV DNA level, with hepatitis Be antigen no longer detectable in four of them. The authors speculated that production of the cytokine IFN-alpha by type 2 dendritic cell precursors in response to HIV infection may well have decreased the HBV DNA level. Interestingly, the decreases in HBV DNA levels were not associated with increased ALT or total bilirubin levels, supporting a role for a noncytopathic cause of the HBV DNA level decrease.

Among liver transplant recipients with HBV and HCV coinfection, HDV infection was associated with the suppression of HCV replication [80]. Furthermore, in a distinct model for HCV superinfection, where both recipient and donor were infected with different HCV strains, detailed genetic analyses showed that only one strain of HCV could be identified at each time point in all cases [81].

5. A new hypothesis proposed to explain the clinical efficacy of the coinfection therapy

The clinical efficacy of IBDV coinfection in hepatitis patients is rather difficult to explain, since the natural hosts of IBDV and HCV (birds and humans, respectively) are separated by the several hundred million years of evolutionary distance.

The family *Birnaviridae* was established in 1986 to describe and classify a group of viruses, which carry a bisegmented double-stranded RNA (dsRNA) genome as their prominent characteristic. The two main representative of this virus family are the infectious pancreatic necrosis virus of fish (IPNV) and the causative agent of infectious bursal disease of chickens. In fact, the IBDV is not known to be a hazard in transmitting to other species despite its worldwide distribution in the domestic fowl, while some zoonotic diseases are of continuing concern [82,83]. The age-dependent sensitivity of chicks towards IBDV infections is determined by the exquisite tropism of IBDV for the lymphoid follicles of the bursa of Fabricius of chickens. The underlying mechanism of such tropism is far from being resolved.

Since both IBDV and HCV are lymphotropic in their natural hosts, it is a compelling speculation that the clinical efficacy of IBDV results from its binding to specific receptors on the CD81⁺ human hepatocytes and B lymphocytes (i.e., the target cells for HCV). If so, IBDV may dominate viral replication during dual infection.

Our hypothesis could be tested in vitro based on the following molecular biology technique. Efficient RNA replication systems for culture-adapted HCV genotypes 1a and 1b have been established in the highly permissive Huh-7.5 hepatoma cell line [84]. In this system it was shown that HCV RNA replication and protein expression can be specifically inhibited by RNA interference. This is a recently discovered antiviral mechanism present in plants and animals that induces double-stranded RNA degradation. The antiviral effect

was found to be independent of IFN. These results suggested that RNAi may represent a new approach for the treatment of persistent HCV infection [85]. Sound physico-chemical and biological considerations (based on substantial data on the interactions of various cell types with proteins-antibodies, viral preparations, accumulated from the 1960s onwards) would 'predict' that IBDV coinfection of the Huh-7.5 hepatoma cell line is likely to inhibit RNA replication of culture-adapted HCV genotypes.

It is the long established commercial practice to produce IBDV live vaccines in the VERO cell line for the poultry industry. The production of IBDV on a large scale is quite straightforward. Without any huge development costs, the proposal for the routine inexpensive production of IBDV for human clinical trials, with special attention to vaccines safety with efficacy for global benefit [86], is likely to be attractive. Additionally, valuable data are becoming available on the adsorption (and possible internalisation) of HCV on VERO cells assessed by quantifying the cell-associated viral RNA by a real-time RT-PCR method [87].

HCV present in human plasma that is able to replicate in cell culture was inoculated on VERO cells or human hepatocarcinoma cells to characterize the two putative HCV receptors, namely, CD81 that interacts *in vitro* with the HCV E2 envelope glycoprotein, and the low-density lipoprotein receptor (LDLr) that interacts with HCV present in human plasma. (There is always a possibility that apart from CD81 and the human scavenger receptor class B type 1 (SR-B1), additional hepatocyte-specific co-factor(s) are necessary for HCV entry [88].)

Anti-LDLr antibody, low and very low density lipoproteins inhibited significantly the adsorption of HCV, confirming the role of LDLr as HCV receptor. Only one out of the two anti-CD81 antibodies used in this study led to a partial inhibition of HCV binding. This paper also highlights a role for glycosaminoglycans (GAGs)² in the adsorption of HCV: treatment of virus with heparin led to 70% inhibition of its attachment, as did desulfation of cellular GAGs. Treatment of VERO cells with heparin-lyase² [EC.4.2.2.7] significantly inhibited virus attachment but by only 30%.

It was reported recently that HCV envelope glycoproteins E1/E2 interact with infections pseudotype retroviral particles and efficiently mediate entry into target cells [89]. Only primary hepatocytes and one hepatoma cell line were susceptible to HCV pseudovirus entry that could be inhibited by sera from HCV-infected individuals. Expression of the putative HCV receptor CD81 on nonpermissive human hepatic but not on murine cells enabled HCV pseudovirus entry. It seems that the HCV attachment to target cells, following successful 'hits' at the cell membrane electrical envelope, presumably, occurring first is involved in the inhibition of

viral entry by an anti-CD81 mAb. The authors conclude that 'CD81 functions as a post-attachment entry coreceptor and that other cellular factors act in concert with CD81 to mediate HCV binding and entry into hepatocytes.'

It is not surprising that there were differences between the interactions of the virus with respect to the putative HCV receptor expressed on nonpermissive human hepatic but not on murine cells that is suggested to enable the entry of the HCV pseudovirus [89]. This is because the two cellular systems possessing different surface topochemistry and the associated electrical properties, would govern differently the respective exquisitely specific cell-virus interactions. This in turn would probably alter and distort the three dimensional structures around the 'gateway' and interact differently for the entry of HCV pseudovirus particles into cells. A 1978 publication in the Proceedings of the Royal Society B [and Ann Immunol (Paris), 1977, IAAI (1979)] had already emphasised that there were striking differences between the cell surface macromolecular architecture even of *H-2^f* and *H-2^s* spleen T cells of mice (and thymocytes, not B or RBCs) of different major histocompatibility haplotypes (or some other genes related to it) [90–92]. In such cases differences in the outcome of interactions for virus entry into cells (with different surface topochemistry and the associated electrical properties) are predictable. An additional striking observation is that the human CD81 (*hCD81*) specifically interacts with its putative receptor HCV, but soluble HCV glycoprotein E2 failed to interact with the African green monkey (VERO cell) CD81 (*AGMCD81*), which differs from *hCD81* at four amino acid residues within the large extra cellular loop (LEL) [93]. Mutation of *hCD81* sequence at each of the four residues corresponding to the sequence of *AGMCD81* identified amino acid 186 to be critical for maintaining an interaction with soluble E2 [94]. This is consistent with the findings in many laboratories that HCV does not replicate in VERO cells (derived from the *AGM* kidney epithelial cells) and because VERO cells possess surface topochemistry (and the related electrical properties) different from those of human cells, the two cell types interact differently.

It seems likely that IBDV coinfection would perhaps inhibit HCV binding and possibly internalisation into VERO cells. Extensive physico-chemical studies [95–98] on the binding of proteins and viruses to cells suggest that IBDV coinfection would, predictably, interfere with and inhibit HCV binding to VERO cells. To what extent would such interference prevent internalisation of the virus into cells would become clear when some experiments, planned in SCID mice carrying a plasminogen activator transgene with chimeric human livers, will have been completed [99]. The biological relevance of the biophysical-electrokinetic aspects governed by the topochemistry of the gene products proteins expressed on the cell surface was discussed recently [100]. Such studies may throw some light on the mechanisms of action and may be carried out using wild-type IBDV strains, and chimeric viruses containing either the determinants for cell-specific replication or cell tropism [101,102].

² Heparin lyase cleaves off polysaccharides containing 1,4-linked glucuronate or iduronate residues and 1,4- α -linked 2-sulfoamino-2-deoxy-6-sulfo-D-glucose residues to give oligosaccharides with terminal 4-deoxy-alpha-D-gluc-4-enuronosyl groups at their non-reducing ends.

5.1. Avian duck hepatitis B virus (DHBV), a possible model of DNA viruses relevant to the human hepatitis B virus for investigating superinfection exclusion mechanism and anti-DHBV drugs interactions.

There is an additional model system for studying the clinically observed efficacy of IBDV coinfection therapy. The *Hepadnaviridae* family contains DNA viruses, such as the human hepatitis B virus, the avian duck hepatitis B virus and the rodent woodchuck hepatitis B virus (WHV). DHBV is distributed in both wild and domestic ducks. DHBV is a safe surrogate for HBV because of their similarities.

Several cell culture systems have been developed to study anti-DHBV drugs and disinfectants [103]. Studies with duck hepatitis B virus as a model demonstrated that the early viral entry steps of hepatitis B viruses into hepatocytes are different from those of other viruses reported so far [104].

It was also suggested that the intriguing phenomenon of superinfection exclusion, wherein a virus prevents the subsequent infection of an already infected host cell, may result from the role of the L surface antigen of DHBV as a regulator of intracellular trafficking [105]. So far, it appears to be restricted to duck hepatitis viruses.

We believe that the DHBV model would also be useful to investigate the exclusion phenomenon in decompensated chronic hepatitis patients with intentional IBDV coinfection (or in HIV patients with natural GB virus-C coinfection). It would be interesting to explore whether dominance by another avian virus would also be mediated by a similar mechanism.

6. Eight out of ten studies reporting a beneficial effect of GBV-C viremia on HIV-related mortality or response to therapy and improved-delayed survival in HIV

Hepatitis G virus (GB virus-C; GBV-C or HGV), causes persistent, non-pathogenic infection in a large proportion of the human population. GBV-C has been classified in the family *Flaviviridae*. The viral genome is a single-stranded, ~9.5 kb long RNA molecule of positive polarity that is translated into a single polyprotein of about 3000 amino acids. GBV-C/HGV is transmitted parenterally and probably sexually. The genome of GBV-C exhibits a sequence variation among different isolates and at least four major genotypes of GBV-C are, type 1 (West Africa), type 2 (US/Europe), type 3 (Asia), and type 4 (Southeast Asia). Epidemiology data suggest that GBV-C infection is present in 8–14.6% of the population in developing countries and in 1–1.4% of the healthy population in developed countries [106].

In the March 4, 2004, issue of NEJM, Williams et al. (Iowa) evaluated 271 men, who were participants in the Multicenter Acquired Immunodeficiency Syndrome Cohort Study for GB virus C (GBV-C) viremia [107]. The authors reported that GBV-C inhibited the replication of human im-

munodeficiency virus in vitro, and concluded that GBV-C viremia was significantly associated with prolonged survival among HIV-positive men 5–6 years after HIV seroconversion, while the loss of GBV-C RNA by 5–6 years after HIV seroconversion was associated with the poorest prognosis. In the June 19, 2004, issue of the Lancet, Xiang et al. provided insight into the epidemiological association between GBV-C infection and longer survival in HIV-infected individuals, demonstrating that GBV-C induces HIV-inhibitory chemokines, and reduces the expression of the HIV coreceptor CCR5 in vitro [108]. Eight of the ten studies devoted to HIV/GBV-C coinfection influences suggest a beneficial effect of GBV-C viremia on HIV-related mortality or response to therapy [109].

Earlier results from Iowa [110] and Hanover [111] relating to the data on a total of 559 patients, receiving treatment for HIV/AIDS, prompted the authors [110,111] to speculate about a possible role of GBV-C infection to treat HIV. Some highly influential authors engaged in developing other concepts (some working closely with commercial companies, albeit declaring financial interests in some of their papers) have expressed scepticism about the usefulness of the intentional coinfection strategy [112].

7. The role of apathogenic viruses for the treatment and control of AIDS and hepatitis

Mother nature's educating example of how the different virus strains could influence the replication of each other in a population is the emergence of rabbit haemorrhagic disease virus (a non-enveloped RNA virus of the class *Caliciviridae*). This virus has killed hundreds of millions of wild rabbits in Australia and Europe, but in the UK there appears to be an endemic non-pathogenic strain, that could dominate over the pathogenic one [113].

One cannot be critical about ultra caution when new treatment strategies are being proposed in the absence of any other option being available. Then, one cannot go wrong. Nonetheless, it was suggested that cautious pilot studies were needed for improving therapy. Because many pharmaceutical giant companies working closely with academics, are developing alternative new treatment drugs (that are bound to be expensive), it would be not only a great pity to discourage serious attempts at doing so, but scandalous.

In the absence of any commitment on our part to a pharmaceutical company, following the Ehrlich and Cambridge traditions, academic and compassionate reasons impel us to encourage cautious trials of the "intentional coinfection strategy" in patients with HIV infection by the use of hepatitis G virus [15,16]. One needs to consider seriously the role of unrelated viruses for the control and treatment of AIDS that are of increasing concern worldwide. The estimated worldwide prevalence of HIV infections topped 52.5 million in June 2003, a mere 20 years after the aetiological agent was shown to be a sexually transmissible virus. More than 22 million

people have died of the acquired immunodeficiency syndrome (AIDS). In one generation the condition and persistent epidemics have become the most devastating in recorded history. The impact of HIV in Africa has been so profound that it influences political, economic, agriculture/food security, social, education, defence, science and health considerations [114]. Although the situation is the worst in sub-Saharan Africa, the fastest growing epidemic is in Eastern-Europe [115] and an HIV-1 epidemic is also being projected to soon explode in the world largest countries, India and China [116]. Both the United Nations and the Chinese government predict ‘China’s Titanic Peril’ with the number of HIV carriers reaching a staggering 10 million by the year 2010 in China alone [117].

IBDV cannot be judged to be a risk to humans since experience, based on very widespread use, has shown that there is no evidence or likelihood of zoonotic transmission. Unlike many pathogenic virus vectors, the avian IBDV poses no danger to the general population. Consistent with this an IBDV preparation was safely and effectively used in a clinical trial for the treatment of 42 acute B and C hepatitis patients [13].

8. Conclusions

The Consensus Conferences on Hepatitis C and National Institutes of Health Consensus Development Conference Statement on the Management of Hepatitis C [118,119] emphasised that IFN-alpha-based treatments are contraindicated in patients with decompensated cirrhosis. All the four of the published cases of successfully treated chronic hepatitis patients discussed here had decompensated hepatitis [14,15]. We list below nine other arguments for critically considering cautious clinical trials of IBDV coinfection strategy for the treatment and control of chronic hepatitis patients:

- (1) We are inclined to make the cautious suggestion that IBDV might be suitable for other hepatitis patients, considering (i) the effectiveness of ‘intentional coinfection therapy by IBDV’ in decompensated hepatitis without serious side effects, and (ii) the recent data from two centres on the prolonged survival of over 300 HIV patients naturally infected with an apathogenic virus. This may be of benefit to the patients, who are not eligible for current therapies, including those with mild disease and normal alanine aminotransferase (ALT) levels, patients with advanced liver disease, children, the elderly, patients with ongoing or recent alcohol and substance abuse, renal disease, severe psychiatric or neurologic illness, autoimmune disorders, solid organ transplant, and other significant comorbid conditions [41].
- (2) Hepatic fibrosis and cirrhosis are generally considered to be irreversible. Surprisingly, recent investigations albeit in three cases seem to suggest [120] that in patients, who respond to antiviral therapy, cirrhosis due to chronic hepatitis B might be reversible. Consequently, to make a beginning, the authors encouraged clinicians to treat all cirrhotic patients with a treatable active underlying disease with appropriate therapy, even in the presence of clinical evidence of decompensation and liver biopsy report of extensive fibrosis or histological cirrhosis. A striking feature of the IBDV therapy noted earlier [15] was the regeneration of the liver and appears to be in satisfactory agreement with this recent report.
- (3) Even the best available treatment, the combination of pegylated interferon and ribavirin, which is costly and fraught with side effects, eradicates HCV in only 50% of patients with genotype 1 infection. (One of the four cases had HCV 1a genotype-induced liver inflammation [15].)
- (4) The risk of IFN-alpha-based treatment failure is markedly increased by the notoriously poor compliance to treatment and adherence. This may be, presumably, because of the side effects of the treatment, including those related to the CNS (depression). (Despite a less than optimal medication adherence, IBDV coinfection treatment did improve the condition of the patients and was associated with long-lasting remission or significant clinical improvement [14].)
- (5) It is likely that the current pegylated IFN-alpha and ribavirin combination therapy already offers the maximum clearance and eradication of the virus that is achievable with these drugs. At present, it seems unlikely that IFN-alpha-based therapy will be replaced rapidly by new drugs for the treatment and control of the disease [121], especially, in the vast populations of the less well off countries.
- (6) Canada set aside \$CDN1.1 billion to compensate HCV patients infected by blood transfusion [122]. Several countries, including Ireland, France, Sweden, and New Zealand, have compensation programs for individuals thought to have acquired HCV infection through the blood supply. Furthermore, the National Health Service in Britain and the Hungarian government have been required to offer compensation to individuals with transfusion acquired HCV infection (as the result of legal judgments). If considered appropriate, approved and agreed, such patients may well elect to undergo IBDV coinfection therapy within the compensation program at a fraction of the costs.
- (7) The total direct health care cost associated with HCV is estimated to have exceeded \$1 billion in 1998. Future projections predict a four fold increase between 1990 and 2015 in persons at risk of chronic liver disease (i.e., those with infection for 20 years or longer), suggesting a continued rise in the burden of HCV in the United States alone in the foreseeable future [123].
- (8) It is estimated that over the next ten to twenty years, complications of cirrhosis, such as hepatic decompensation and hepatocellular carcinoma will double in number, and deaths caused by liver disease may nearly triple [124].

(9) During the past two years following the increase in the waiting lists, and a large number of patients on the waiting lists dying, adult-to-adult living donor liver organ transplantation (ALDLT) has increasingly been carried out in Europe and in the USA [125]. It seems fatuous to have to wait for new drugs and put the health of living donors at risk when the safe and efficient IBDV coinfection therapy is already available.

We realise that the IBDV coinfection therapy was tested only in four well documented cases of chronic decompensated hepatitis patients [14,15].

Suggesting a serious debate for cautious trials of IBDV (of proven safety) albeit in four patients with decompensated hepatitis is not quite so extraordinary considering the success of treatment in (i) only one patient before the start of routine cord blood stem cell therapy, (ii) in two patients before extending trials of Prusiner treatment for vCJD, and (iii) in three cancer patients treated with the Baylor cancer vaccine.

It is essential to make these ideas known for encouraging the creation of conditions to extend studies for collecting more data. Over the centuries as with several other useful pilot studies on new treatments, such as even Ehrlich's Salvarsan to treat syphilis, a beginning had to be made before they became routine therapies. After all, ribavirin, in combination with interferons, has proved clinically useful for the treatment of hepatitis C virus (HCV) infection despite uncertainty as to its true mechanism of action [126]. Here we wish to reiterate [16] that it would be a great pity to discourage cautious trials of IBDV in chronic decompensated hepatitis. In the absence of any treatment available and the success of IBDV, it is frustrating to have to wait for new drugs and put the health of living donors at risk. The development of coinfection therapy could save lives of many people with no option available at present because many of the patients will die before receiving an orthotopic liver transplantation, when a therapeutic option, such as the coinfection strategy, could be available to all of them.

Note added in proof

Since the submission of the paper, interesting findings about 'Interfering vaccines' in connexion with influenza A virus were published in the 13 August 2004 issue of this journal. Quite comparable to the IBDV coinfection strategy to treat hepatitis, HIV/AIDS discussed above, Noble et al. [127] concluded as follows.

"The interfering vaccine, as demonstrated here with influenza A virus, is a new paradigm. It is a novel, intranasally administered, non-infectious virus preparation, which has a dual antiviral activity. Firstly, it interferes intracellularly with the replication of a lethal dose of wild-type virus, and prevents clinical disease. Secondly, it converts the potentially lethal infection into a sub clinical infection, which stimulates a solid homologous immunity" [127].

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