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Hepatitis C virus (HCV): ever in reliable partnerships?

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Hepatitis C virus (HCV) is a parenterally transmitted hepatotropic pathogen. HCV infection is a major health problem worldwide, frequently causing cirrhosis and liver cancer. There is no preventive vaccine against HCV and treatment, consisting of interferon alpha plus Ribavirin, is generally effective in less than 50% of cases. HCV has evolved mechanisms for surviving in the host. Infection with multiple different HCV variants, as well as interaction with concurrent pathogens, might be successful strategies for viral persistence. The present review illustrates the current status of HCV co-infection with highly relevant pathogens. Issues regarding tropism, disease progression and antiviral treatment response, among other aspects, are discussed. Data accumulated reveal that HCV co-infection should not be considered the mere sum of several independent infections. Some significant questions are still unanswered. Therefore, nowadays, it might be more reasonable to face HCV co-infections as a new biological, clinical and even predominant epidemiological entity.

Key words: Hepatitis C virus, hepatitis B virus, human immunodeficiency virus, co-infection.

INTRODUCTION

Hepatitis C virus (HCV) is a single stranded RNA virus, classified into the Hepacivirus genus, belonging to the *Flaviviridae* family (Mayo and Pringle, 1998). The viral genome encodes a polypeptide precursor consisting of about 3010 amino acid residues, which is cleaved by host and viral proteases (Manabe et al., 1994) to produce structural (core, E1, E2) and non-structural proteins (NS2, NS3, NS4A/B and NS5A/B) with various enzymatic activities. More than 170 million people are infected with HCV worldwide. Most infections with this pathogen are persistent, frequently causing cirrhosis and liver cancer. At present, there is no available preventive vaccine against HCV infection and the consensus therapeutic treatment, consisting of pegylated interferon-alpha (pegIFN-alpha) in combination with Ribavirin, is poorly effective against some viral genotypes (Inchauspe and Feinstone, 2003).

Essential information regarding immune correlates of protective/therapeutic responses against HCV infection is still lacking. Indeed, prevalence of persistent infection indicates that HCV has evolved several mechanisms to evade host immunity, including direct action on cells of the immune system. Particularly, HCV has a high mutational rate, and at least six major genotypes have been defined on the basis of nucleotide sequences of conserved and non-conserved regions. Additionally, coexistence of multiple HCV isolates from different genotypes, or HCV with other pathogens, in the same individual complicates viral infection. In concurrent infections, viral replication and disease progression are likely to be quite different with respect to any of the individual infections.

The aim of the present paper is to review the current status of HCV concurrent infection with highly relevant pathogens. Significant issues regarding incidence, pathogenicity, ways of transmission, antiviral treatment response and disease progression are discussed. Moreover, influence of viral heterogeneity and superinfection with different HCV genotypes, in tropism, host immune response, and several aspects of viral behavior, is analyzed.

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MULTIPLE INFECTIONS BY DIFFERENT HCV GENOTYPES

Being a positive-stranded RNA virus, HCV shows a significant genetic heterogeneity among isolates. In this respect, at least six major genotypes have been described so far (Simmonds et al., 2005). Several studies report multiple infections by different genotypes and/or subtypes in the same individual (Laskus et al., 2001; Preston et al., 1995; Roque-Afonso et al., 2005). All these studies refer to high risk groups. Nevertheless, Giannini and co-workers demonstrated that infections by more than one HCV genotype may actually occur despite the absence of any particular risk factor (Giannini et al., 1999).

It seems that in most cases seroconversion and ongoing viremia do not guarantee protection against re-infection. Thus, a new viral strain, even belonging to the same HCV genotype or subtype of the resident strain, can surmount the immune response directed at the latter, and rapidly become the dominant quasispecies (Herring et al., 2004).

This fact poses a key question: Are multiple infections by different HCV genotypes/subtypes a cause of a clinically relevant modification of the natural course of HCV-related hepatic or extrahepatic manifestations?

In this respect various authors (Herring et al., 2004; Schijman et al., 2004) agree in that no correlation is observed between superinfection and viral load. Whether the genotypes replicate with equal efficiency is not clear (Schijman et al., 2004). While some authors have found no clinical differences between co-infected patients and those with a single infection (Giannini et al., 1999), others describe an acute exacerbation of the chronic disease (Accapezzato et al., 2002; Kao et al., 1993). In hemophiliacs, multiple changes in HCV genotypes were observed in 58 % of the subjects, over a 3–15-year period of observation. There was no obvious trend toward replacement of any particular HCV genotype. Some of these patients had a subsequent reappearance of the genotype that had been replaced. Remarkably, genotype changes occurred while the subjects, who had previously received untreated clotting-factor concentrates, were receiving only virus-inactivated concentrates, implicating reactivation rather than re-infection (Eyster et al., 1999).

The analysis of three cases of HCV-infected recipients, who received blood from one or two HCV-infected donors, revealed that all donor strains established infection, while the recipient strain became undetectable, even as a minor sequence. In both cases that received blood from two different donors, one of the donor strains took over and the other was detectable only transiently, and as a small minority of the circulating strains (Laskus et al., 2001). Whether the amount of virus introduced during transfusion determines the outcome of superinfection cannot be deduced from those results, as in all three analyzed cases the donor strain overtook the

recipient strain, regardless of the viral load in the donor (Laskus et al., 2001). Besides, as Laskus and colleagues emphasize, the overall amount of virus in the recipient, taking into account the amount of virus in body fluids and the liver, must have been much larger than the amount transfused in 1 U of blood (Laskus et al., 2001).

On the other hand, in five HCV-infected recipients of livers from HCV-infected donors, the recipient strain prevailed, whereas in other six, the donor strain took over. In each of the cases, the donor or the recipient strains were detected only transiently, early after transplantation. The takeover of one strain by another seemed to be complete and long lasting, because the displaced strain remained undetectable for up to 50 months of follow up (Radkowski et al., 2001). Whether the immune system plays any role in the selection of infecting strains is unclear. Actually, the fact that the immune system was unable to eradicate the infection before transplantation argues against the possibility that it would mount an efficient response in the setting of pharmacological immunosuppression (Radkowski et al., 2001). Thus, it seems far more likely that the outcome of superinfection is determined by direct competition between infecting strains. In this respect, Radkowski and coworkers (Radkowski et al., 2001) consider that the Competition Exclusion Principle, which states that in the absence of niche differentiation, one competing species will eventually eliminate or exclude the other, may apply to viral infections in humans. This is so because the evolution of higher fitness variants is a frequent event in viral populations.

There is mounting evidence that peripheral blood mononuclear cells (PBMC) could represent an independent viral compartment in which any variant strain, with mutations that favor its tropism for these kinds of cells, could avoid the competing pressure exerted by the dominant serum strain. In a study conducted by Roque-Afonso and colleagues (2005) nine out of 116 patients had in their PBMC a strain assigned to a genotype different from the genotype detected in plasma. Transplantation and intravenous drug use appeared significantly and independently correlated to the existence of compartmentalized variants. In two transplant patients, plasma and PBMC harvested before (1 and 6 years before the index sample) and after transplantation, showed the same genotypic compartmentalization (Roque-Afonso et al., 2005). Other authors have found that viral populations changes, in the setting of liver transplantation from HCV-infected donors to HCV-infected recipients, occurred simultaneously in PBMC and serum (Radkowski et al., 2001).

The issue of HCV replication in extrahepatic sites, such as PBMC, could have implications for the development of strategies targeted at preventing recurrent infections. A study conducted in HCV-positive transplant recipients of liver from a HCV-negative donor, suggests that the liver graft is primarily colonized by liver-derived virus

remaining in the circulation. However, virus variants of extrahepatic origin can be detected in serum early after transplantation, but they were undetectable already at 1 month post transplant (Laskus et al., 2002).

The eradication of one strain by another could have potential implications for novel treatment options. In this respect, Radkowski and colleagues (2001) think it is possible to exploit viral competition for therapy, since this has been already demonstrated for influenza A virus infections. Nevertheless, it can always be argued that the displaced strain can exist and continue to replicate at levels under the detection limit, even of direct sequencing. The reported unreliability of commonly used genotyping techniques for the detection of mixed infections supports this doubt; particularly, genotyping methods based on PCR amplification of conserved regions, with universal primers, may fail to detect sequences present in very low concentrations (Giannini et al., 1999). It is worldwide known that to achieve high sensitivity, large numbers of clones must be sequenced, which is a highly laborious, expensive and time consuming task. This makes the rigorous execution of these assays unaffordable for routine exams.

Moreover, Roque-Afonso and co-workers report that PBMC specific virions were not detected in the circulation of co-infected patients (Roque-Afonso et al., 2005). Therefore, they speculate, not only that those variants could have been present in plasma at an undetectable level, but also that a cell-to-cell transmission could occur without the need for cell-free virions. In any case, if they had not assayed PBMC, only serum samples, they would not have been able to detect the mixed infection. Following this same line of thinking, it is possible that HCV can replicate in other still unknown extrahepatic sites in the same specific manner. This could cause that those variants never reach plasma, so are they being ignored so far? If this is true, the actual prevalence of multiple infections by HCV is being underestimated, not only because of techniques shortcomings, but also because of limited information about possible viral tropism. Therefore, taking into account the state of the art and the knowledge gained so far, we do not consider possible that the complete takeover or suppression of one strain by another can be undoubtedly affirmed. On the other hand, and seemingly in open contradiction, PCR-based genotyping methods may also result in an overestimated frequency of this phenomenon due to pitfalls such as lack of primer specificity (Giannini et al., 1999).

The need to improve genotyping methods in terms of reliability and practicability is of paramount importance due to HCV genotype-dependent treatment recommendations. For example, an intravenous drug user (IVDU) whose infecting strain was determined to be of subtype 3a on two occasions, failed to respond to the recommended 6 months antiviral therapy regimen with peg-IFN-alpha and Ribavirin. Therefore, the genotype

analysis was repeated, revealing subtype 1b. A recent re-infection could be excluded because the patient had stopped intravenous drug use (Schroter et al., 2003). These results provide evidence that minor strains, which respond less favorably, might influence the outcome of therapy.

HCV-HBV CO-INFECTIONS

HCV and hepatitis B virus (HBV) are very different viral entities (Rehermann and Nascimbeni, 2005). However, both viruses, although using different mechanisms of immune escape and survival strategies, can cause persistent infections leading to chronic liver disease, including very severe and lethal damage (Rehermann and Nascimbeni, 2005). These two pathogens are responsible for 80% of primary liver cancer worldwide (Bosch et al., 2004). The prevalence of patients with HCV-HBV co-infection has been described as high in geographic areas where a high endemic level of both infections is reported, such as Western Asia and the Mediterranean Basin (Sagnelli et al., 2000; Tsatsralt-Od et al., 2005). One Eastern European study found a rate of dual infection in 0.68% of a randomly selected healthy population (Atanasova et al., 2004). In general, the interaction between HCV and HBV has been poorly investigated. Particularly, information regarding clinical presentation in HCV-HBV co-infected individuals is very scarce. Some relevant data are now slowly accumulating.

HCV and HBV infect humans parenterally, having similar sources of transmission (Crockett and Keeffe, 2005). No facilitation of viral transmission has been associated with one of these pathogens with respect to the other. HBV-HCV acute concurrent infection is a very rare event (Chu and Liaw, 1995; Mimms et al., 1993). HBV replication generally comes first and has already cleared when HCV replication becomes evident. Whether HBV active replication delays HCV serum expression should be more extensively studied. These patients generally recover from HBV infection but develop chronic hepatitis C (Sagnelli et al., 2002).

Acute HBV infection in chronic HCV infected individuals is commonly detected in countries where drug addicts, non-vaccinated against HBV, are numerous (Coppola et al., 1994; Esteban et al., 1989). HBV infection in HCV chronic carriers is associated with a marked inhibition of the HCV genome as shown by the absence of HCV-RNA in serum during the acute phase of the illness. In these cases, HBV tapered off in a few weeks, and half of the patients remained HCV-RNA-negative for at least 6 months and the other half became HCV-RNA-positive with a low HCV viral load (Sagnelli et al., 2002). Interestingly, HBV viral load and dynamics of HBV-DNA negativization in HBV-acutely infected individuals seems to be unaffected by concurrent HCV chronic infection. Further investigation is needed to evaluate if the more

rapid seroconversion to anti-HBeAg observed in these patients reflects a partial inhibition of HBV replication exerted by a pre-existing HCV chronic infection (Sagnelli et al., 2002).

On the other hand, there are some case reports indicating that HCV superinfection suppresses a pre-existing HBV chronic infection (Guido et al., 1998; Liaw et al., 2000; Wietzke et al., 1999). Inhibition of HCV replication by HBV in HCV acute-HBV chronic infection is not clear (Chu et al., 1999a; Chu et al., 1999b; Romano et al., 1995; Sagnelli et al., 2005; Sagnelli et al., 2006). Considering all these facts together, the sequence of acute infections seems to be an important factor in viral interaction.

Patients with chronic HBV-HCV concurrent infection show a reciprocal inhibition of viral genomes. In fact, HBV infection not only inhibits HCV replication alone, but also, and all the more so, when associated with hepatitis D virus (HDV). The prevalence of patients with HCV RNA in serum is significantly lower in HBV-HCV or HBV-HDV-HCV infected individuals with respect to HCV infection alone (Sagnelli et al., 2000). Prevalence of patients with circulating HBV e-minus strain was much lower in HBV-HCV co-infection than in those with HBV infection alone, although no difference has been observed with respect to the circulating HBV wild strain. Several explanations are possible for this phenomenon: the inhibitory effect of HCV on HBV might limit the emergence of precore variants because the lesser the viral genomes replicate, the fewer mutant genomes will be created; the immune system, weakened by the presence of multiple infections, is less efficient in exerting a selective pressure for precore variants; or HCV might exert a greater inhibitory effect on the precore variants than on the wild-type viruses because the former may have a lesser capacity to replicate. Remarkably, Rodriguez-Inigo and colleagues have recently shown that hepatitis C virus (HCV) and hepatitis B virus (HBV) can coexist in the same hepatocyte. HBV DNA and HCV RNA levels in co-infected hepatocytes were lower than those in cells infected only with the respective virus (Rodriguez-Inigo et al., 2005).

An association with a severe clinical presentation and an infrequent response to interferon alpha treatment has been observed in patients with chronic HBV-HCV concurrent infection (Sagnelli et al., 2000; Sagnelli et al., 2001a; Sagnelli et al., 2001b). Particularly, it has been demonstrated a synergism of HCV and HBV infection in causing hepatocellular carcinoma and lethal damage (Bosch et al., 2004; Rodriguez-Vidigal et al., 2003). The observation that HBV-HCV concurrent infection, whether acute or chronic, and HBV superinfection in HCV chronic carriers are characterized by a severe clinical presentation stresses the need for HBV vaccination in all HBsAg negative chronic hepatitis patients (Sagnelli et al., 2002).

Interestingly, patients with HCV-RNA positive chronic

hepatitis who are HBsAg, anti-HBsAg negative, but anti-HBcAg positive seem to have circulating HBV DNA, and more frequently a severe liver disease and poor response to interferon alpha treatment than HCV-RNA positive patients lacking all HBV markers (Cacciola et al., 1999; Zignego et al., 1997). These patients may be at the last step in a hypothetical sequence of events leading to a progressive suppression of HBV replication during HBV-HCV chronic co-infection (Sagnelli et al., 2005; Tanaka et al., 2004). The mechanism leading to more severe forms of liver damage in these patients might be explained by the fact that hepatocytes with a low level silent HBV replication might be a suitable target for cytotoxic T cells previously sensitized to viral antigens. In fact, in chronic hepatitis B, cell-mediated attack is frequently strong in cases of low to moderate exposure of viral antigens on the hepatocyte membrane and weak in cases of a high expression. Immunoreaction might bring about an increased inflammation and fibrosis regardless of the detectability of circulating HBV-DNA. Moreover, the hepatocellular apoptosis observed in HBsAg, anti-HBsAg negative, but anti-HBcAg positive individuals might be an expression of HBV silent infection (Sagnelli et al., 2005).

This kind of "silent infections" could be more frequent than previously thought, and some apparently single infections might really be concurrent HCV-HBV infection. In fact, an important portion of subjects present dynamic virological profiles characterized by fluctuation of HBV and/or HCV viremia levels that at different time points are over or under the cutoff limits (Raimondo et al., 2005). Consequently, a correct diagnosis could be performed in these subjects only by serially repeating the virological tests 1 year apart. Longitudinal evaluation is also very important for tailoring the appropriate therapeutic schedule in co-infected patients.

Hepatitis B vaccination is less effective in patients with advanced liver disease, especially after decompensation, such as in patients awaiting liver transplantation, and in liver transplant recipients. The emerging lower rates of inherent immunity in younger individuals, higher morbidity and mortality of acute hepatitis B superimposed on chronic liver disease, and greater vaccine efficacy in milder forms of chronic liver disease suggest that it is a reasonable policy to recommend hepatitis B vaccination in patients early in the natural history of chronic liver disease (Crockett and Keeffe, 2005).

There is no currently established standard of care for patients who are co-infected with HBV and HCV. In general, the same treatment criteria should be applied to patients who are HBV-HCV dually infected as are applied to mono-infected patients. Initiation of treatment, as with both HBV and HCV single infections, is recommended in patients with active chronic hepatitis or cirrhosis, prior to decompensation. Care must be taken to select the most appropriate antiviral regimen, based on serologic markers and levels of viremia, since exacerbations of liver disease after initiation of therapy have been described, likely due

to loss of viral suppression from the successfully treated dominant virus. Because of its activity against both viruses, interferon therapy has been the most studied, alone and combined with Ribavirin and/or Lamivudin. The assessment of the "dominant" virus is helpful in determining a treatment strategy (Crockett and Keeffe, 2005). In the near future, the ability of HCV core protein to inhibit HBV gene expression and replication (Chen et al., 2003), as well as to modulate immune response to hepatitis B surface antigen (Aguilar et al., 2003), could be used for rational development of therapeutic or preventive treatments against these viral agents.

HCV/HIV CO-INFECTIONS

HCV and human immunodeficiency virus (HIV) are both transmitted parenterally. This shared route of transmission makes HCV co-infection rate be, in HIV infected subjects, as high as 92% (Backmund et al., 2005) or even 100% (Ruan et al., 2004) in certain risk groups. HIV, classified as a lentivirus of the Retroviridae family, is not hepatotropic as is HCV. Nevertheless, there are evidences of still not fully understood mutual interactions between HIV and HCV, which clinical, biological and epidemiological consequences require further assessment. In this respect, Flichman and colleagues found a transitory clearance from plasma of HIV-RNA after HCV superinfection in a patient previously infected by HIV. Therefore, they postulate that HCV superinfection may have temporarily interfered with HIV replication (Flichman et al., 1999) although it cannot be ruled out the possibility of a spontaneous decrease of HIV viral load below the detection level of PCR. These authors hypothesize that this phenomenon might only occur during the first stages of HCV infection when it is usually sub-clinical and any change in HIV viral load is not suspected of being produced by HCV superinfection. Alternatively, immunological host responses triggered by HCV superinfection, such as cytokines production, might influence the viral dynamics of HIV replication (Flichman et al., 1999). The interpretation of these results is obviously highly speculative, as long as this was observed in only one patient. Other large cohort studies must be designed to draw out clear conclusions in this respect. The influence of the infection sequence on viral interference, if any, must be addressed too. No case of inhibition of HCV replication by HIV has been reported so far.

Generally, authors find higher HCV RNA levels in the co-infected patients compared to HCV infected subjects (Alatrakchi et al., 2005; Eyster et al., 1999; Goedert et al., 2001; Yee et al., 2000). Yee and colleagues consider that this reflects the inability to contain the virus in the face of immunosuppression (Yee et al., 2000). At the same time, HIV seroconversion was associated with a 4-fold increase in HCV RNA levels in hemophiliacs (Eyster et

al., 1999). In these patients HCV genotypes` changes are more frequent when they are co-infected by HIV; and HCV genotype replacement is associated with a 30-fold steeper increase in viral load over time (Eyster et al., 1999). The clinical implications of increased viremia are not thoroughly understood, but its significance in transmissibility has been documented (Granovsky et al., 1998).

Regarding vertical transmission, it seems likely that HIV co-infection enhances HCV transmission (Granovsky et al., 1998); maternal HCV infection is associated with increased HIV vertical transmission too (Hershow et al., 1997). Although the statistical power of these studies is usually limited by the small number of studied patients, there are some evidences of increased risk of transmission of either virus when maternal viral load of the other is high (Granovsky et al., 1998; Hershow et al., 1997). Likewise, HCV co-infection appeared as a significant predictor for HIV infection in IVDU (Backmund et al., 2005). At the same time, it has been reported that the risk for sexual transmission of HCV infection tends to increase in those who are HIV-infected among non-IVDU (Giuliani et al., 1997), as well as among IVDU (Soto et al., 1994). However, none of these studies could address whether each virus influences directly the transmission of the other, or if they are only markers for other confounding factors.

Possibly, the facilitation of HCV transmission by HIV, in any scenario, could be explained by the higher HCV viral load observed in co-infected patients, as has already been discussed earlier in this paper. Or it could also be hypothesized that immunosuppression allows or favors the development of highly infectious HCV variants, which otherwise would be under control. At the same time, the known negative effect of HCV in the immune status of infected subjects, particularly the chronicity-favoring cytokine environment, might also impact HIV replication somehow, facilitating the emergence of variants with increased infecting capacity. It is also reasonable to consider that the presence of both viruses in the same individual represents a stronger pressure that fuels the selection of such variants. Another important issue that should not be forgotten is the possibility that a previous infection with any of these viruses could render the patient more susceptible to acquire a second infection. This could be plausible, either because of immune deterioration, or due to other still unknown factors that demand further study.

Some evidences encourage taking into account HCV genotype when evaluating the relationship between HIV and HCV as a potentially important factor. Although the number of patients was limited, stratification by genotype yielded the suggestion that an inverse correlation between HIV and HCV viremia time courses may be associated with genotype 4 (Caudai et al., 2005). Similarly, the risk of end stage liver disease with HCV-HIV co-infection appeared to be 2-fold higher with

genotype 1 than with genotype 2 or 3 (Goedert et al., 2001).

Due to the improvements in life expectancy gained by HIV infected patients since the introduction of highly active antiretroviral therapy (HAART), HCV-related complications are now a major cause of death in co-infected patients. The introduction of HAART led to a significant reduction in the rates of opportunistic infections (Klein et al., 2003), death (Klein et al., 2003), hospitalization (Klein et al., 2003) and in the risk of fulminant hepatic failure (FHF) (Kramer et al., 2005) among HIV singly infected individuals, but HCV-seropositive subjects have not derived benefit from these reductions (Klein et al., 2003); and in the case of FHF, an opposite trend was observed with respect to both HCV and HIV mono-infections (Kramer et al., 2005).

Thus, the impact of each virus, on the spectrum of clinical manifestations of the other, remains as an issue of paramount importance. According to Cheng and colleagues (1999) the degree of renal insufficiency in HIV-infected patients with HCV-related glomerular disease (HCV-GD) is more advanced than reported for HCV mono-infected patients (Cheng et al., 1999). Conversely, hypocomplementemia and/or cryoglobulinemia showed a lower prevalence than reported in HCV-GD without HIV co-infection (Cheng et al., 1999). Interferon alpha treatment did not prevent the development of renal failure, nor did it prolong survival, while interferon alpha therapy in HIV-negative patients with HCV-GD induced a significant reduction in proteinuria (Cheng et al., 1999). Pineda and colleagues' results showed that HIV co-infection shortens markedly the survival of patients with HCV-related cirrhosis after the first decompensation (Pineda et al., 2005). The frequency of hepatic encephalopathy, as both the first decompensation and the cause of death, was higher in HIV-co-infected patients. These investigators considered that HIV may lead to increased hepatocyte apoptosis, which could worsen liver fibrosis. At the same time, some hepatotoxic antiretroviral drugs may enhance its progression too. On the other hand, hepatocellular carcinoma (HCC) was much more frequent in HIV non infected patients; in this respect the authors hypothesize that this may be the result of the shorter survival of HCV-HIV co-infected subjects (Pineda et al., 2005).

Regarding mental health, HIV-HCV co-infected patients seem to perform worse neurocognitively on tests of executive functioning and are also more likely to be diagnosed with HIV-associated dementia (Ryan et al., 2004). Thus, these authors consider that HCV-infected leukocytes can carry the virus into the central nervous system where viral replication may be sustained in an independent compartment. Decompensated liver functioning may result in cognitive dysfunction (hepatic encephalopathy) too, either through reduced extraction and metabolism of encephalopathic substances or portosystemic shunting (Ryan et al., 2004).

On the other hand, in patients with AIDS-related lymphoma, no relation was found between HCV infection and lymphoma histology or site (Levine et al., 1999). Possible reason for this is that the latent period between initial HCV infection and lymphoma is longer than co-infected individuals' life expectancy. Or, is it possible that HCV is associated with malignancies in some settings other than the context of underlying HIV infection? Surprisingly, Monto and coworkers found that mean steatosis score was lower in co-infected than in mono-infected patients, and the proportion of co-infected patients with no steatosis was higher (Monto et al., 2005). It is important to bear in mind that, in this cohort, previously described steatosis risk factors (e.g. high body mass index, type 2 diabetes mellitus, and genotype 3a infection) were less prevalent in co-infected than in mono-infected patients. In co-infected patients, fibrosis score, age, an undetectable HIV viral load and HAART for more than 24 months were associated with steatosis (Monto et al., 2005). It seems likely, that undetectable HIV RNA is a surrogate for HAART use, and that it is HAART regimen that plays a role in steatosis (Monto et al., 2005). While Giordano and colleagues found there was no increase in the incidence of cirrhosis in the HIV-only group from the pre-HAART to the HAART era, in co-infected patients the rate of cirrhosis increased approximately 20-fold and the risk of HCC, approximately 5-fold (Giordano et al., 2004). These authors suggest that HAART accelerates the development of cirrhosis in the setting of HCV infection, perhaps as a result of toxicity or immune reconstitution. In this respect, Kottlilil and colleagues think that patients with progressive liver inflammation and fibrosis will often be unable to tolerate antiretroviral drugs because of drug-related hepatotoxicity (Kottlilil et al., 2004).

No clear guidelines are available concerning which infection should be treated first. Uberti-Foppa and colleagues found that anti-HCV pretreatment is independently associated with a decrease in severe liver toxicity as the result of a subsequent antiretroviral regimen (Uberti-Foppa et al., 2003). The patients who had never been treated for HCV infection had a significantly higher risk of being unable to tolerate anti-HIV drugs, and the protective effect of anti-HCV therapy was maintained after the discontinuation of treatment. The only subjects receiving anti-HCV pre-treatment, who discontinued antiretroviral drugs, were those who did not achieve a sustained HCV virologic response (Uberti-Foppa et al., 2003). On the other hand, some authors consider that co-infected patients under HAART can be safely treated for HCV with pegIFN alpha-2a alone or in combination with Ribavirin, without a negative impact on specific HIV parameters (Khalili et al., 2005). They found that adverse effects during therapy did not appear to be more frequent or severe than those observed in HCV mono-infected patients. The late addition of Ribavirin lacked of serious complications too. At the same time,

other investigators emphasize that didanosine use, as part of HAART, should be avoided when treatments for HCV and HIV have to be concomitantly administered. Interactions between Ribavirin and didanosine are a major cause of hepatic decompensation (Bani-Sadr et al., 2005b) and mitochondrial toxicity (Bani-Sadr et al., 2005a; Laguno et al., 2005).

Although female sex is considered a risk factor for developing grade 4 liver enzyme elevations (Wit et al., 2002), when trends in aminotransferase levels were stratified by type of antiretroviral therapy (ART), French and colleagues results showed that, among women who continued to receive protease inhibitors (Pis) or nonnucleoside reverse transcriptase inhibitors (NNRTIs), there were decreases or no significant changes in aminotransferase values over time, respectively (French et al., 2004). These findings support the observation that continued ART does not lead to continued elevation of aminotransferase levels. Mean levels did not increase with HAART initiation either, and actually decreased (French et al., 2004).

The presence of HIV seems to influence HCV tropism since the negative strand HCV RNA, indicative of active viral replication, was detected at higher rates in the PBMC compartments of HIV-HCV co-infected patients (Blackard et al., 2005). The mechanism behind this phenomenon is not clear. It seems unlikely that this results from viral interference of HIV on HCV because there was no correlation between plasma HIV RNA levels and positive- or negative-strand HCV RNA levels in either the serum or PBMC compartment. An inverse correlation between CD4+ cell counts and positive-strand HCV RNA levels was found in the serum, but not the PBMC, compartment (Blackard et al., 2005). This might be considered another evidence of the independent nature of PBMC as a replication compartment for HCV. It is also possible that HIV, through the induction of interferon antagonists, blunts host innate antiviral responses that would otherwise inhibit HCV replication. HIV may also render specific types of PBMC more susceptible to HCV infection and replication (Blackard et al., 2005).

Results regarding the impact of these two persistent viruses on the immune system are conflicting. Matthews-Greer and colleagues have observed a significant inverse relationship between HCV RNA load and CD4+ T-cell count (Matthews-Greer et al., 2001), while Stebbing and coworkers' analyses did not show any differences in CD4+ cell count decreases between HIV-infected patients and patients with HIV-HCV co-infection (Stebbing et al., 2005). There were no observed differences between HCV+ and HCV- subjects in the proportion obtaining a CD4+ response with HAART and HCV+ subjects experienced similar reductions in HIV RNA (Klein et al., 2003). Concomitant HIV infection does much younger ages than the HIV-co-infected (Granovsky et al., 1998).

According to Stebbing and colleagues' results, the allogeneic or primary stimulatory capacity of dendritic cells (DC) derived from HIV-HCV co-infected individuals is similar to that observed in DC from HIV-only infected individuals (Stebbing et al., 2004). These authors did not find significant differences between levels of DC costimulatory molecules in the matched groups either. These data suggest that HCV does not play a direct role in altering HIV disease progression or in subverting the responses of HIV infected DC. Therefore, DC derived immunotherapeutic approaches being developed for HIV could also be used in co-infected individuals. Alatrakchi and colleagues compared the intrahepatic T cell responses between the HCV-HIV co-infected and HCV singly infected patients (Alatrakchi et al., 2005). Despite HIV-related immunodeficiency, there was no difference in the HCV-specific CD8+ or CD4+ cell response. HCV-specific responses appeared to compartmentalize to the liver, as they do in HCV infection alone (Alatrakchi et al., 2005). But the scope of this study is limited by the fact that all co-infected patients had undetectable HIV loads and relatively high, although not normal, CD4+ cell counts due to ART. Therefore, it could not be addressed whether the same results would be found in persons with profound immunodeficiency. Conversely, the comparison of the intraindividual cellular immune response toward HIV and HCV in co-infected persons shows that an individual having a strong cellular immune response against HIV, generally does not have a similar response against HCV (Lauer et al., 2002). It seems unlikely that HIV influence relies solely in the general failure of the immune system, because the majority of the individuals had no evidence of advanced immunodeficiency, as most of them were controlling HIV, either spontaneously or with HAART (Lauer et al., 2002). Another remarkable finding of this study shows that HIV specific proliferative response appears to be more broadly directed in the co-infection rather than in the mono-infection setting. In this respect, the investigators speculate that HCV is having a positive effect on HIV specific immune response, but that this is usually masked by liver disease complications (Lauer et al., 2002). The effectiveness of this diversified response against HIV is still to be accurately assessed. Certainly, larger cohorts of mono- and co-infected individuals need to be studied to address the distinct mechanisms employed by these viruses to evade immune control.

HCV CO-INFECTIONS WITH OTHER PATHOGENS

Several pathogens have also been found in individuals infected with HCV, in spite of differences in ways of transmission, life cycle and course of disease (Table 1). Some of them clearly influence HCV infection and clinical course. For instance, patients co-infected with HCV and the trematode *Schistosoma mansoni* have an increased

Table 1. Summary of some relevant non-HBV/HIV-HCV co-infections.

Pathogen	Incidence in HCV infected patients	Influence	References
TTV	High in some Asian countries	Neither severity of liver disease nor replication and genotype distribution of HCV was affected by concurrent TTV infection.	- (Perardi et al., 1999) - (Dai et al., 2003) - (Hsu et al., 2003) - (Liwen et al., 2002) - (Nishizawa et al., 2000) - (Yoshida et al., 2000) - (Kao et al., 2000) - (Yuki et al., 1999)
Hepatitis A virus (HAV)	Low, mainly related to HAV outbreaks into intravenous drug users	High fatality rate, probably due to severe underlying liver damage	- (Spada et al., 2005) - (Vento 2000)
<i>Schistosoma mansoni</i>	Common in Egypt and other developing countries	Increased persistence and severity of HCV infection	- (Farid et al., 2005) - (el Kady et al., 2004) - (el Kady et al., 2005) - (Kamal et al., 2000) - (Kamal et al., 2001) - (Kamal et al., 2004)
Human parvovirus B19	Frequent	Poorly studied. Long-term longitudinal studies are required to determine whether co-infection affects the natural history of chronic hepatitis C.	- (Hsu et al., 2005) - (Lee et al., 2002)
Epstein-Barr virus	Low	Accelerate the course of disease in B-cell NHL patients.	- (Libra et al., 2005)
<i>Helicobacter</i> species	High in patients with cirrhosis and liver cancer	Association between the presence of <i>Helicobacter</i> species DNA in the liver and hepatitis C cirrhosis	- (Rocha et al., 2005)
Herpes simplex virus (HSV)	Low, detected in high risk population	HCV facilitates HSV-2 seropositivity.	- (Singh et al., 2005)
SENV virus	Low, SENV has a specific link to HCV genotype 2a	No apparent effect.	- (Dai et al., 2004) - (Tangkijvanich et al., 2003) - (Kao et al., 2003)
Oral lichen planus	Low	No apparent effect.	- (Campisi et al., 2004)
Human T lymphotropic virus type I and II	Low	A synergistic effect on incident liver disease as well as on death from liver cancer.	- (Hisada et al., 2003) - (Boschi-Pinto et al., 2000)
Hepatitis G virus (HGV)	High	No apparent effect.	- (Lopez et al., 2003) - (Pereira et al., 2002) - (Ling et al., 1998) - (Yu et al., 2001) - (Quintero et al., 2000)

incidence of HCV persistence and accelerated fibrosis. This might be explained because *Schistosoma mansoni* provokes a systemic bias to Th-2 immunity, reducing IFN gamma secreting HCV-specific response (Farid et al., 2005). Recent studies have suggested that bacterial co-infection with *Helicobacter* species, in patients already

infected with HCV, could be involved in the development of cirrhosis and HCC (Rocha et al., 2005). Indeed, the presence of *Helicobacter* species in the liver can be a consequence of structural changes in this organ (intrahepatic shunts) when cirrhosis occurs, and once there, these bacteria can produce toxins which may

interfere with hepatic cells and further contribute to cirrhosis (Rocha et al., 2005). On the other hand, HCV infection may cooperate with Epstein - Barr virus (EBV) infection during progression of B-cell non-Hodgkin's lymphoma (NHL) in immunocompetent individuals. Such an interaction may accelerate the course of disease in B-cell NHL patients (Libra et al., 2005). Besides, HCV facilitates seropositivity to herpes simplex virus (Singh et al., 2005).

Remarkably, some pathogens are particularly frequent partners of HCV in general or in particular populations or geographic areas, without apparent consequences. These are the cases of transfusion transmitted virus (TTV) (Hsu et al., 2003; Liwen et al., 2002; Nishizawa et al., 2000) and hepatitis G virus (HGV) (Ling et al., 1998; Lopez et al., 2003; Pereira et al., 2002). Indeed, the whole picture of epidemiological data suggests that most individuals infected with HCV have been also infected during important periods of time with at least one different pathogen. In the near future, unknown possible interactions between HCV and different pathogens might arise. Only then, a better understanding of HCV infection would be probably available.

FINAL CONSIDERATIONS

Due to the elevated world prevalence of HCV, as well as other parenterally transmitted pathogens, co-infections are a frequent and significant health problem. Nevertheless, demonstrated or potential inhibition of one virus by the other, in the co-infection setting, points out at the possibility of underestimating the actual co-infection frequencies. The facilitation of the transmission of one virus by the other is also an aspect of paramount importance, which is still to be ascertained. Reciprocal interaction between viral entities, specially the influence of other viruses on HCV tropism, as well as the interplay between different HCV genotypes, is a matter of concern due to its implications in recurrent infections. There are still many unanswered key questions in this respect: Are PBMC-specific strains irreversibly adapted to these cells, so that they cannot infect the liver even in the absence of a dominant circulating virus? Is a serum/liver-viral RNA-negative, PBMC-viral RNA-positive individual still a potential source of HCV infection? Therefore, multiple infections complicate the already complex treatment scenario and make clinical outcome hard to predict and manage. There is an urgent need for longer follow up studies, in which larger numbers of high-risk and non high-risk patients are included. The achievement of more sensitive and practical diagnostic methods will shed light into this riddle too.

In light of these elements, would it not be more reasonable to face HCV co-infections as a new biological, clinical and even predominant epidemiological entity? If it is so, each co-infection should not be considered the

mere sum of several independent infections, but rather, a qualitatively different phenomenon in which novel properties emerge. Consequently, HCV co-infections demand a new mentality in terms of diagnosis, treatment design and epidemiological control.

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