**Hepatitis C virus and lipid metabolism: their implications in vaccine development and treatment**

Santiago Duenas-Carrera

Hepatitis C Department, Center for Genetic Engineering and Biotechnology, CIGB
Ave 31 / 158 and 190, ZP 10600, Havana, Cuba
E-mail: santiago.duenas@cigb.edu.cu

**ABSTRACT**

The hepatitis C virus (HCV) infects over 170 million people worldwide and is a leading cause of chronic hepatitis and severe forms of liver damage as cirrhosis and hepatocellular carcinoma. There is no vaccine available against this pathogen and the current therapeutic option, based on the combination of pegylated interferon plus Ribavirin, is expensive, produces undesirable side effects, and is effective in approximately half of the patients treated. HCV establishes a complex and not completely understood interaction with the host. In addition to its variability and interference with the immune system function, the HCV life cycle is closely associated with lipid metabolism and this relationship contributes to viral persistence. The present review analyzes the current state of the art in this association and the disturbances generated, mainly expressed as intracellular lipid accumulation in hepatocytes and increased oxidative stress with negative consequences in the immune response. Moreover, the potential impact on the development of vaccines and more effective therapeutic interventions against this virus, in the context of the disorders in lipid metabolism, is discussed. Finally, perspectives for rational intervention, taking into account the dependence of HCV to lipid metabolism, and potential targets, are evaluated.

**Keywords:** HCV, vaccine, VLDL, lipid, therapy

**RESUMEN**

**Virus de la hepatitis C y metabolismo lipídico: implicaciones para el desarrollo de vacunas y tratamientos.** El virus de la hepatitis C (VHC) infecta a más de 170 millones de personas globalmente y es la causa principal de hepatitis crónica y formas graves de daño hepático, como cirrosis y carcinoma hepatocelular. No existe vacuna disponible contra este patógeno y la terapia actual que se basa en la combinación de interferón pegilado más Ribavirina provoca efectos secundarios y solo es efectiva en aproximadamente la mitad de los pacientes tratados. El VHC establece una compleja interacción con el hospedero que aún no ha sido completamente caracterizada. El ciclo de vida del VHC se relaciona estrechamente con el metabolismo lipídico, lo que junto a su variabilidad genética e interferencia con el funcionamiento del sistema inmune contribuye a la persistencia viral. En esta revisión se analiza el estado del arte de tal interacción, así como las alteraciones que provoca, fundamentalmente la acumulación de lípidos en los hepatocitos y el incremento del estrés oxidativo, con la consiguiente afectación a la respuesta inmune. Además, se discute el impacto potencial para el desarrollo de vacunas e intervenciones terapéuticas contra el VHC en el contexto de un metabolismo lipídico alterado. También se abordan las perspectivas para una intervención racional de la infección, teniendo en cuenta la dependencia del VHC en el metabolismo lipídico y los blancos potenciales de tales procedimientos.

**Palabras clave:** VHC, vacuna, VLDL, lípido, terapia

**Introduction**

Hepatitis C virus (HCV) infection is a worldwide health problem, causing chronic hepatitis in approximately 85% of the cases, with a frequent progress to severe forms of liver damage like cirrhosis and hepatocellular carcinoma [1]. HCV is a parenterally transmitted pathogen that frequently induces extra-hepatic disease expressions such as essential mixed cryoglobulinemia and membranoproliferative glomerulonephritis [2, 3]. There is no vaccine currently available against this pathogen, and therapeutic treatments, based on pegylated interferon (PegIFN) plus ribavirin are expensive, produce undesirable side effects and are only effective in about one half of the patients [4]. Successful response to treatment against HCV infection seems to depend on several factors, involving both, the virus and the host [5-7].

HCV is a single positive strand RNA virus belonging to the Flaviviridae family, hepacivirus genus. The HCV genome encodes a polyprotein co- and post-translationally processed in at least ten viral proteins with different roles in viral pathogenesis [8]. Recent advances in HCV cell culture replication have enhanced the knowledge on the HCV life cycle, although the complete picture is yet unknown. However, one thing is clear, the virus and host establish a very complex interaction during infection. HCV heterogeneity and mutability, as well as a deficient immune response to this pathogen are perhaps the most relevant factors of viral persistence. Six main genotypes have been described for HCV, with important differences in aspects such as response to standard treatment [9]. Individuals infected with genotype 1 have the worst response. On the other hand, there is evidence that HCV can replicate in, or at least enter into, cells of the immune system, in addition to the hepatocytes [10]. In fact, several immune system mechanisms, both the innate and adaptive responses, related to the potential clearance of HCV infection, are affected in the chronic phase with: increased resistance to interferon, defects in the function of antigen recognition, viral evasion of the immune system, and new immune mechanisms induced by viral infection [11].
presenting cells and natural killer cells, specific T cell impairment and exhaustion, among others [11, 12]. HCV core and E2 proteins have been frequently associated to these effects, although other viral proteins also seem to be involved [13].

On the other hand, although life style is sometimes underestimated, it is relevant for HCV-related disease and treatment outcomes. In addition to alcohol and drug use, patients should avoid the excessive intake of sugar and fat-enriched food. Particularly, liver steatosis, defined as excessive accumulation of lipid in the cytoplasm of hepatocytes, is a frequent histological feature in HCV chronically infected patients [14]. In vivo and in vitro studies have indicated that HCV could alter intrahepatic lipid metabolism by affecting lipid synthesis, oxidative stress, lipid peroxidation, insulin resistance and the assembly and secretion of very low density lipoproteins (VLDL) [15-18]. The degree of liver steatosis and insulin resistance has been negatively associated to therapy response [19, 20]. The strong relationship of HCV and lipid metabolism seems to involve every step of the HCV life cycle bringing up many still unanswered questions. The present review will analyze different aspects of the relationship between HCV and lipid metabolism, and discuss their potential implications in the development of efficacious preventive and therapeutic interventions.

HCV life cycle and lipids

It is well known that HCV circulates in the host as a quasispecies, a population of genetically related molecules differing at the nucleotide level [21]. In addition, virion particles of different sizes and density have been detected in circulation [22]. The existence of particles lacking HCV E1-E2, or completely non-enveloped, has also been described in patients [23]. The detection of HCV genomic mutants, mostly lacking HCV E1-E2, or completely non-enveloped, is also described in patients [23]. In addition, other HCV genome mutants, mostly lacking HCV E1-E2, or completely non-enveloped, have been described in patients [23]. It is well known that HCV circulates in the host as a quasispecies, a population of genetically related molecules differing at the nucleotide level [21]. In addition, virion particles of different sizes and density have been detected in circulation [22]. The existence of particles lacking HCV E1-E2, or completely non-enveloped, has also been described in patients [23]. The detection of HCV genomic mutants, mostly lacking HCV E1-E2, or completely non-enveloped, has also been described in patients [23].

The degree of liver steatosis and insulin resistance has been negatively associated to therapy response [19, 20]. The strong relationship of HCV and lipid metabolism seems to involve every step of the HCV life cycle bringing up many still unanswered questions. The present review will analyze different aspects of the relationship between HCV and lipid metabolism, and discuss their potential implications in the development of efficacious preventive and therapeutic interventions.

Table 1. Main points of connection between HCV life cycle and lipid metabolism

<table>
<thead>
<tr>
<th>Step of HCV life cycle</th>
<th>Viral proteins involved</th>
<th>Lipid metabolism counterpart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulation in the bloodstream</td>
<td>Structural proteins (Core, E1 and E2)</td>
<td>LDL, HDL, LDL, chylomicrons, others?</td>
</tr>
<tr>
<td>Entry</td>
<td>Structural proteins (Core, E1 and E2)</td>
<td>LDL-R, SR-BI, plasma membrane lipid composition, others?</td>
</tr>
<tr>
<td>HCV replication</td>
<td>NSSA replication complex (probably all HCV proteins)</td>
<td>Geranylgeranyl lipid and different lipid components of intracellular membranous web</td>
</tr>
<tr>
<td>HCV morphogenesis and virion release</td>
<td>Structural proteins (Core, E1 and E2) and probably NS3/4A and NSSA</td>
<td>VLDL assembly pathway (lipid droplets, ApoA, ApoE, MTP, lipids)</td>
</tr>
</tbody>
</table>


19. Santiago Dueñas-Carrera HCV and lipids: prevention and therapy


Indeed, there is strong evidence suggesting that some HCV proteins, particularly the core and NS5A, can induce hepatic steatosis by interfering with intracellular lipid metabolism [36]. Two main predominant forces of steatosis have been proposed to coexist in patients with hepatitis C. The first is a metabolic type that is seen mainly in HCV-1 infected patients and is associated with increased body mass index, hyperlipidemia, and insulin resistance. The second is a viral type that may also be developed in the absence of any other steatogenic factors and that seems to be directly triggered by the virus through the interference with intracellular lipid metabolism or the induction of insulin resistance [36]. These two forces are not mutually excluding but probably synergic in generating hepatic steatosis in hepatitis C patients.

Impact on vaccine development

Different elements support the rationale of generating an effective vaccine against HCV. The most relevant fact is probably that immunity to the virus can be produced since 20-30% of individuals exposed to the virus spontaneously clears the infection and the immune system is critical to this outcome [28]. On the other hand, in HCV chronic infections, immune response is not only unable to clear the virus but also seems to contribute to liver damage and extra-hepatic disease expressions [11, 37]. Therefore, strategies based on the specific immune modulation, including vaccination strategies are truly promising. However, the generation of vaccines against HCV has become a challenging task.

The resolution of HCV infection requires a complex interplay between innate and adaptive immune responses. In the absence of suitable animal models, only chimpanzees are consistently infected by HCV but major ethics and cost-related reasons have limited its use. Studies using samples from individuals spontaneously eliminating HCV infection have shed light on immunological correlations with HCV clearance. It is generally supported that a strong, multispecific and sustained T-cell response seems to be required for viral clearance. Strategies, generally targeting different HCV antigens, have been evaluated for developing preventive or therapeutic vaccines against this pathogen. In fact, several vaccine candidates have undergone clinical evaluation [38-40]. These vaccine candidates were found to be safe and immunogenic [41-43]. However, the clinical and virological impact of these vaccine candidates must be demonstrated.

The goal of vaccination against HCV can be seen in three different scenarios: protection or complete clearance of HCV; control of infection avoiding the development of liver damage; the promotion of favorable conditions in the patient for a more effective response after anti-viral treatments. The first setting is ideal but it is undoubtedly the most difficult one to achieve. In fact, the scenario in vaccine development has changed in the last decade, with early studies focused on preventive vaccination and current strategies mainly addressing the therapeutic approach. Hence, most vaccine candidates under clinical evaluation have been designed to elicit cell-mediated immune response. Different factors have led to a decrease in the number of ongoing preventive vaccination studies against HCV. Scientifically, the absence of a complete definition of immunologic parameters correlating with protection and/or the clearance of HCV, and particularly the controversial role of neutralizing antibodies, are probably the most important elements related to this situation. In favor of antibody response, subjects with primary hypogammaglobulinemia showed rapid disease progression and poor response to interferon treatment [44]. Moreover, previous studies reported the presence of antibodies specific to E2 HVR in individuals who spontaneously resolved HCV infection [45, 46]. However, there is relevant data on the null or delayed induction of neutralizing antibodies in HCV infection [47, 48]. Additionally, since at least some neutralizing antibodies are directed towards HVR-I, the induction of this type of response has been involved in selecting viral diversity and a mechanism for viral escape. In other cases, neutralizing antibodies cross-reacting with HCV isolates from different genotypes have been found in chronically infected HCV patients, indicating a high degree of conservation of the targeted epitope [49, 50]. Nevertheless, these antibodies, even when induced at high levels, are unable to clear chronic HCV infection [49].

The heterogeneity and mutability of HCV are particularly important for the viral escape from the immune system and persistence. In the light of current knowledge, the association of HCV with lipoproteins poses an additional negative impact for the effective induction and action of neutralizing antibodies. HCV particles may be attached to or incorporated into VLDL during the assembly of the lipoprotein particles and secreted together with VLDL. The nature of the association between HCV and VLDL remains unclear. If HCV hides in the core of VLDL, as suggested [22], it makes the virus unique in that the entire virion is not exposed to the serum during circulation. Obviously, if this is the situation, neutralizing antibodies targeted at viral epitopes will not be effective against circulating HCV particles. As previously explained [26], this scenario does not necessarily contradict the observation that the entry of cell culture infectious HCV was inhibited by antibodies targeting viral structural protein E2, since these antibodies may also be included in endocytic vesicles containing HCV. In fact, it has been reported that immunoglobin G can enter clathrin coated pits and action of neutralizing antibodies. HCV particles may hide completely in VLDL because it reduces the sensitivity of HCV to neutralizing antibodies [52-53] could occur through an increased presence of BSI in the cell membrane, thereby reducing exposure time to interferon alpha-2b and ribavirin combination therapy in Japanese patients with chronic hepatitis C. Hepatol Res. 2006, 35:19-25.


to neutralizing antibodies. In any case, if this partial exposure scenario is correct (according to the nature of the association between HCV and VLDL, which is not completely understood), the viral regions exposed may not always be the same in HCV particles. This could be an additional source of viral heterogeneity. Moreover, this might be a viral mechanism to circumstantially disfavor the exposure of relevant immunogenic or neutralizing epitopes.

According to the state of the art on the association between HCV and VLDL, a vaccine against this pathogen designed to generate neutralizing antibodies should target several epitopes at the same time. Additionally, the most relevant epitopes could be conformation-dependent and this conformation may require a lipoprotein context. Moreover, important epitopes could even share regions of both HCV proteins and the VLDL structure itself. In a further degree of complexity, since the composition of VLDL is not always exactly the same, a greater variability is thence expected. Therefore, vaccine candidates involving liposome or lipid moieties in general may be advantageous, although thorough studies are required since there is a risk of inducing or enhancing auto-immune disorders from this manipulation.

On the other hand, in persons that are overweight and/or have baseline liver steatosis and other dysfunctions of lipid metabolism, there were side effects [54, 55] related to oxidative stress, persistent inflammation, disturbance in the signaling cascade of interferon and down-regulation of its receptors, reduction in the signaling cascade of interferon and down-regulation of its receptors seems to be the main mechanism interfering with the treatment, mainly (although not exclusively) by increasing oxidative stress [54, 62]. Interestingly, individuals infected with genotype 3 HCV isolates respond differently (better) to PegIFN plus ribavirin than those infected with genotype 1 isolates [4]. This may be due to several causes. Noteworthy, these genotypes, as previously stated, have been described as differently behaving in relation to the predominant hepatic steatosis driving forces and molecules involved in lipid metabolism, mainly due to differences in the HCV core proteins of these genotypes [36]. It has been recently demonstrated that the management of dismetabolism, diet and exercise therapy can improve the body mass index, liver histology and, therefore, the response to PegIFN and Ribavirin [63]. Since HCV-related alterations of lipid metabolism are supposed to increase with years of infection, the early treatment of patients eligible for therapy with PegIFN plus ribavirin is advised. Therefore, in time, some undesirable co-morbidities caused or enhanced by lipid accumulation and dysfunction, such as heart disease and hypertension, could also worsen the results after therapy or even become contraindications to treatment.

New therapeutic agents specifically targeting HCV proteins mainly involving undesirably beneficial effects in lipid metabolism like nucleosapid and NS5A could be advantageous. In fact, most vaccine candidates of ongoing clinical trials in HCV-infected individuals target at least one of these antigens. Interestingly, CIGB-230 (a vaccine candidate based on a recombinant HCV core protein co-administered with a plasmid expressing the HCV core, E1 and E2 proteins) elicited predominantly a cell-mediated immune response against the HCV core when administered to HCV genotype 1b patients who were unresponsive to previous treatments with IFN plus ribavirin [38]. Liver damage was also reduced in a subset of those patients.

Genetic background has been previously correlated with protection against chronic hepatitis C virus infection [60]. Remarkably, ApoE-containing lipoproteins have the ability to modulate key elements of the immune response by either inhibiting or stimulating antigen and mitogen induced T-lymphocyte activation as well as proliferation [61]. In fact, ApoE interacts with signals from multiple mitogens including transferrin and interleukin 2 (IL-2), and its impact on the pathology of infectious diseases like hepatitis C, has been linked with its immunomodulatory properties [60].

**Implications for drugs therapy**

Nowadays, the best therapy is the weekly administration of PegIFN (1.5 μg/kg for PegIFN-alfa-2a or 180 μg/kg for PegIFN-alfa-2a) and the daily ingestion of ribavirin (1000 mg for body weight below 75 kg and 1200 mg for body weight above 75 kg), for six months (in HCV genotypes 2 and 3), or one year (in HCV genotypes 1 and 4). Not all HCV-infected patients are eligible for the treatment. A successful therapy is that of a sustained virological response, established by undetectable HCV RNA levels, six months after the end of the standard treatment. Patients who achieve such a response usually show an improvement in liver histology and clinical outcomes [4].

As previously stated, overweight and liver steatosis has been found to be independent factors for non-response to therapy in patients infected with HCV after the treatment with PegIFN plus ribavirin [19]. In this case, the disturbance in the signaling cascade of interferon and down-regulation of its receptors seems to be the main mechanism interfering with the treatment, mainly (although not exclusively) by increasing oxidative stress [54, 62]. Interestingly, individuals infected with genotype 3 HCV isolates respond differently (better) to PegIFN plus ribavirin than those infected with genotype 1 isolates [4]. This may be due to several causes. Noteworthy, these genotypes, as previously stated, have been described as differently behaving in relation to the predominant hepatic steatosis driving forces and molecules involved in lipid metabolism, mainly due to differences in the HCV core proteins of these genotypes [36]. It has been recently demonstrated that the management of dismetabolism, diet and exercise therapy can improve the body mass index, liver histology and, therefore, the response to PegIFN and Ribavirin [63]. Since HCV-related alterations of lipid metabolism are supposed to increase with years of infection, the early treatment of patients eligible for therapy with PegIFN plus ribavirin is advised. Therefore, in time, some undesirable co-morbidities caused or enhanced by lipid accumulation and dysfunction, such as heart disease and hypertension, could also worsen the results after therapy or even become contraindications to treatment.

New therapeutic agents specifically targeting essential components of the viral life cycle, such as the HCV NS3/4A serine protease and NS5 RNA-dependent RNA polymerase, are currently in advanced clinical development [64, 65]. Interesting results concerning increased sustained virological response, when combined with PegIFN plus ribavirin, has been obtained in clinical practice with some of these molecules [66]. However, HCV mutant isolates resistant

to these molecules have been described and toxicity is not always low [67]. Since all intracellular steps of the HCV life cycle in hepatocytes seem to be associated to the membranous structure and depend on the association of viral proteins with lipid droplets and lipoproteins, molecules targeting polyprotein processing and RNA replication could be also interfered by the lipid environment.

The strong relationship between HCV and lipid metabolism has opened new gates in the search for therapeutic interventions since, for instance, drugs that target cholesterol metabolism may be useful in treating HCV infection. Results show that the treatment of cells with statins (the widely used cholesterol lowering drugs) inhibits HCV RNA replication by depleting geranylgeranyl lipids [68]. However, applying statins to treat HCV will require very high doses and would likely cause toxicity in the liver and other organs [26]. Other types of drugs used for treating hypercholesterolemia by blocking the assembly and secretion of VLDL, have been found to inhibit the production of HCV particles from infected cells [69]. Some of these molecules, i.e. antisense RNA drugs targeting ApoB and several MTP inhibitors, have already been tested in clinical trials [68]. Particularly, a long-term treatment with MTP inhibitors led to the toxic accumulation of fat in the liver.

Conclusions

HCV life cycle and lipid metabolism are connected. Therefore, the rational manipulation of this relationship emerges as a potential strategy for developing preventive and therapeutic interventions against HCV infection. Nevertheless, there must first be a complete definition of the molecular mechanisms governing that relationship. Knowledge on the exact HCV particle architecture and composition is crucial for vaccine development, particularly (but not exclusively) for those strategies designed to elicit neutralizing antibody responses. In the therapeutic setting, the realization of a multifactorial approach with less-toxic anti-cholesteroleomics and immunomodulators, in addition to safer anti-virals, should play a more significant role to eliminate liver and extra-hepatic expressions of HCV infection. Last but not the least, there are the efforts required for population awareness on healthier life styles which, together with scientific achievements for creating effective vaccines or medicines against HCV, will be critical for successful interventions against this pathogen.

Acknowledgements

The author would like to acknowledge M.Sc. Yalena Amador for the critical reading of the manuscript.

Received in August, 2010. Accepted for publication in February 2011.