Review

Containing “The Great Houdini” of viruses: Combining direct acting antivirals with the host immune response for the treatment of chronic hepatitis C

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Abstract

Presently the development of new therapies for hepatitis C virus (HCV) is rapidly moving forward. Almost every week new data appear on how direct acting antivirals (DAAs) succeed or fail in clinical trials. Despite the potency of many of the DAA combinations, the effect exerted by ribavirin (RBV) is still needed for an effective therapy in many new DAA combinations. Due to the strong antiviral effect of DAAs, it is likely that a major complementary therapeutic effect exerted by RBV is immune modulation resulting in an increased barrier to development of resistance. For HCV genotype 1a infections elimination of pegylated interferon, is not possible in many DAA combinations without jeopardizing the results. The host immune response is thus likely to play a key role even during DAA-based therapies. Hence, T cells may recognize and eliminate viral variants with resistance to the DAAs. We herein show several examples where this may be the case, supporting the rationale of including the host response also in the new therapeutic regimens. This review will describe the potential benefits of combining various DAAs with means to activate the specific immune response against HCV.

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1. Introduction

The development of therapies for chronic infections caused by the hepatitis C virus (HCV) has exploded in the past five years with the introduction of direct acting antiviral (DAA) compounds
(Hofmann and Zeuzem, 2011; Welsch et al., 2012). Pegylated interferon alpha 2a, or 2b (IFN) and ribavirin (RBV) is presently still used as a backbone when combined with the 1st generations protease inhibitors (PIs) against HCV (McHutchison et al., 2009). The efficiency of the combination is dependent on the host IL-28B genotype (CC, CT, or TT), the viral genotype (gt) 1–6, and the viral load (Ge et al., 2009). The IL-28B genotype also predicts the chance to achieve spontaneous resolution of an acute HCV infection (Thomas et al., 2009). By using baseline factors a prediction of sustained viral response (SVR) can be done with moderate accuracy. In addition, the kinetics of the early viral kinetics during therapy has been found very useful for the prediction of SVR and has generated stopping rules during therapy when a low probability for eventual final cure is at hand (Sherman et al., 2011). This reduces overtreatment and unnecessary treatment and reduces the cost and adverse events in patients who will have a low chance to achieve SVR and hence be taken off therapy.

IFN/RBV treatment does not cause emergence of viral resistance and the mechanisms for non-response to IFN/RBV is poorly understood. Viral strains which do not respond to IFN therapy with an at least 2 log decline during the initial 12 weeks treatment could be defined as resistant to IFN/RBV and are defined as null responders (Wedemeyer et al., 2012a). No specific mutations have been associated with such resistance. However, identification of an interferon sensitivity-determining region (ISDR) has been published in Japanese patients (Enomoto et al., 1996). With respect to RBV no specific viral genotype or phenotype resistance has been identified.

This strongly contrasts to what is seen with the direct acting antivirals (DAAs; Fig. 1), where resistance mutations are readily detected in the target protein, which explain the lack of efficacy once they occur (Welsch et al., 2012). Identification of viral resistance mutations, hence, can be expected to play a role during monitoring of treatment with new DAA-based combination therapies. New treatment strategies with combination of several DAA compounds targeting different regions of HCV will be used to overcome emergence of resistance if such combinations will be sufficient or if addition of immune modulating therapies will be needed in difficult to treat patients remains to be explored (Figs. 2 and 3).

1.1. The mechanisms of action of IFN and RBV

After having acknowledged that the combination of IFN and RBV can cure around 50% of the patients with chronic genotype 1 (gt1) HCV infection and such 80% of genotype non-1 infections, the question arises how these drugs act on the infected cell. With respect to IFNβ, it is well known that it binds to the IFNβ/β receptor (IFNAR), which is composed of the two subunits IFNAR1 and IFNAR2, constitutively expressed on the surface of many cells including hepatocytes. The binding of IFNβ to its receptor results in the activation of the Janus kinases Jak1 and Tyk2, which phosphorylate signal transducer and activator of transcription (STAT) 1 and 2. STAT1 and 2 form a complex with the IFN-regulatory factor 9 (IRF9), which binds to IFN-stimulated response elements (ISRE) on DNA leading to the expression of several hundred genes named IFN-stimulated genes (ISGs). These ISGs have a variety of antiviral, antiproliferative and immunomodulatory effects. Some of them such as the protein kinase R (PKR) or the 2′-5′ oligoadenylate synthetase (OAS) directly inhibit viral transcription and translation, and thus, reduce virus replication, and others act by strengthening the antiviral immune response. Hence, IFNα is known to be involved in the induction of T cell proliferation, the activation of NK cells, the maturation of dendritic cells and the prevention of T cell apoptosis (Pitha and Kunz, 2007).

Successful IFNs treatment is characterized by two phases (Neumann et al., 1998). In the first phase, a rapid decline of the viral load is believed to be caused by a direct antiviral effect exerted by IFN (Neumann et al., 1998). The antiviral effect is mediated by ISGs such as the PKR or the 2′-5′ OAS, which reduce the viral replication by directly inhibiting viral transcription and translation. The second, and slower viral decline phase is thought to be immune mediated by the IFN/RBV stimulation of innate and adaptive immune system. IFNα is e.g. known to be involved in the induction of T cell proliferation and cytotoxicity (Le Bon et al., 2008a,b), the activation of NK cells (Trinchieri and Santoli, 1978), the maturation of dendritic cells (Le Bon et al., 2001) and the augmentation of B cell responses (Le Bon et al., 2001; Badr et al., 2010).

RBV on the other hand has a much less well characterized effect on the infected cell. Several direct or indirect mechanisms have been proposed. RBV is well known to deplete the cell of the guanosine tri-phosphate (GTP) necessary for viral RNA synthesis by blocking the enzyme inosine-5′-monophosphate dehydrogenase (IMPDH) (Malinoski and Stollar, 1981). Furthermore, RBV has been proposed to act as an inhibitor of the RNA-dependent RNA polymerase (Maag et al., 2001), and as a mutagen causing an error catastrophe (Crotty et al., 2000). The high concentrations needed for RBV to act as a direct antiviral agent causes major adverse events and cannot be used in practice, and are difficult to reach in vivo (Zoulim et al., 1998). Hence, the effect of ribavirin during HCV therapy is likely to be immune modulatory e.g. by altering the Th1/Th2 balance towards an antiviral Th1 response (Hultgren et al., 1998; Ning et al., 1998), or by inducing the expression of ISGs (Liu et al., 2007; Zhang et al., 2003). Even today when using new highly potent DAA drugs RBV seems to be needed as a complement to increase the efficacy via a presumed immune modulatory effect which is not provided by the current DAAs.

It is important to note that the most refractory patients to IFN/RBV combination therapy are those with ISG already switched on (Sarasin-Filipowicz et al., 2008). The continuous activation of ISGs by intracellular HCV RNA in these patients is not sufficient to clear the virus but results in an upregulation of negative regulators in the Jak-STAT pathway such as protein inhibitor of activated STAT (PIAS) 1 and suppressor of cytokine signalling (SOCS) 3 causing a decrease in the sensitivity to IFNα. Patients lacking an ongoing IFN response before therapy, who do not have upregulated ISGs, will have a strong ISG induction and activation of an intracellular IFN necessary for the treatment to be effective.

An important finding linking the host immune response to treatment outcome was the identification of the IL-28B (IFNA3) single nucleotide polymorphisms (SNPs) (Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009). It was shown that patients with genotype (rs12979860 CC or rs8099917 TT) had much higher SVR rates than patients lacking this polymorphism. Interestingly, hepatic ISG expression before treatment was initiated was found to be significantly lower in patients with the favourable IL28B genotype (Honda et al., 2010). IFN/RBV combination therapy in these patients resulted in a strong ISG induction and a better treatment response with higher SVR rates.
As IFNα, IFN beta, and IFNα activates the Jak-STAT pathway resulting in the expression of ISGs through different receptors. The IFNα receptor is expressed only in specific cell types such as hepatocytes, epithelial cells, bone marrow cell lines, and plasmacytoid dendritic cells (Sommerreyns et al., 2008). IFNα is characterized by a potent antiviral activity and less pronounced side effects since it does not bind to bone marrow cell lines. Due to this it is currently tested in clinical phase 3 trials (Muir et al., 2010; Zeuzem et al., 2011).

1.2. The mechanisms of action of the major classes of DAAs

Before 2011 the only available treatment for HCV, was consisting of pegylated IFN and RBV. Now two compounds from the first generation protease inhibitors, Boceprevir (Merck) and Telaprevir (Janssen, Vertex Pharmaceuticals), has been approved for use in man. More effective DAAs from different classes such as polymerase inhibitors, NS5A inhibitors, and second generations PIs are under fast development and expected to be available within the next few years, which will improve treatment substantially (Fig. 1). Already 2003 the non-structural (NS)3/4A protease inhibitor (PI) BILN2061 was tested during two days and shown to effectively reduce the HCV replication (Lamarre et al., 2003). The development of BILN2061, however, was terminated due to toxicity. This was the start of the DAA era for treatment of HCV. Presently we have two approved NS3 PIs, Boceprevir and Telaprevir, both less potent than BILN2061 but with less pronounced but still significant adverse events (Zeuzem et al., 2011; Poordad et al., 2011). When either of these two PIs is added to the IFN/RBV combination therapy the SVR rate increases from 45% to 75% in patients infected by HCV gt1 strains (Zeuzem et al., 2011; Poordad et al., 2011). Even though the response rates have been improved, and for a majority of patients have allowed shorter treatment duration, the triple combination is complicated and associated with cumbersome adverse events, and a substantial pill burden. Moreover, the currently approved 1st generation PIs works poorly in non-gt1 infected patients.

The second generation PIs with TMC435 (Simeprevir, Medivir, Janssen) and Asunaprevir (BMS) first in line are more potent and have less pronounced side effects and can be taken once daily. TMC435 has been shown to reduce HCV RNA levels by 2–4 log(10)IU/mL and is effective for genotypes 1, 2, 4, 5 and 6, but not for gt 3. It is also better tolerated than the first generations PIs (Moreno et al., 2012). As for Asunaprevir, several studies have demonstrated rapid and substantial decrease in HCV RNA levels in patients with gt1 chronic HCV infection in particularly gt 1b infections (Lok et al., 2012; Pasquinelli et al., 2012). The major mechanism of action for the protease inhibitors is to sterically block cleavage of the HCV polyprotein. The protease inhibitors are generally peptidomimetics simply resembling the natural substrate for the NS3/4A protease. A further additive effect is the inhibitory effect on the cleavage of the host proteins MAVS and TRIF, both signal transducers in the cellular interferon response to the presence of double stranded RNA (Gale and Foy, 2005), and the TC-PTP protein responsible for activation of transcription factors needed for HCV replication (Brendorfer and Sallberg, 2012).

While most focus has been directed against the serine protease active site of NS3, new classes of DAAs are under development and evaluated in clinical trials (Figs. 1–3). There are several compounds targeting the NS5A protein. The NS5A inhibitors bind to the NS5A protein with a function still not fully understood. NS5A has been suggested to be involved in the formation of the membraneous web whereby a blocking of this function would disturb the HCV replication. Daclatasvir (BMS–790052, Bristol-Myers Squibb; Fig. 1) is a promising NS5A inhibitor with a high antiviral potency but a low resistance barrier without a backbone of IFN/RBV treatment (Gao et al., 2010; Nettles et al., 2011; Pol et al., 2012). It is highly effective when combined with the NS5B nucleoside analogue GS–7977 (Sofosbuvir, Gilead). This combination has been suggested to result in a high proportion of cure rates in early clinical trials. Sofosbuvir has shown very promising results in combination with IFN/RBV for gt1 or with only RBV for gts 2 and 3 (Gane et al., 2013; Jacobson et al., 2013; Lawitz et al., 2013). Another promising NS5A inhibitor is GS–5885 (Gilead) that is currently evaluated in a several different DAA containing regimens (NS3, NS5A, NS5B) with or without RBV (http://www.natap.org/2012/EASL/EASL_32.htm). Also Abbot has several DAAs in development, a ritonavir boosted PI (ABT–450/r), a NS5A inhibitor (ABT–267), and a non-nucleoside analogue (ABT–333) which in combination with or without RBV have shown potent antiviral activity and SVR rates over 90% also with treatments as short as 8–12 weeks, as recently reported at AASLD in Boston 2012 as a late breaker poster number 1.
2. The mechanism(s) of immune modulation

2.1. Vaccines

One way to treat a HCV infection is to utilize vaccines that aim at inducing strong and multifunctional CD4+ and CD8+ T cell responses. This type of immune response is found in patients that spontaneously clear their HCV infection but is lacking in patients with established chronic infection (Diepolder et al., 1995; Penna et al., 2007). The need for a therapeutic vaccine can be questioned when the introduction of highly effective DAA drugs with SVR rates approaching 90% have come on the market. Difficult to treat patients groups, however, who needs alternative additive treatments will still exists. For such groups, therapeutic vaccines may be an option, preferably used in combination with DAs, the old SOC (IFN/RBV) or together with other immune modulating agents. The mechanism by which immune modulation is achieved with therapeutic vaccines is thought to be a successful reactivation of the dysfunctional HCV-specific T cell response present in individuals with a chronic HCV infection. This in turn will improve the chance for SVR with any subsequent or parallel DAA treatment regimen. An immune reactivation can be accomplished by using different vaccine compositions. The following vaccine combinations have been used in pre-clinical and clinical studies: recombinant protein/s, peptides, viro- scape peptide formulations, DNA, DNA-recombinant proteins, adenovirus vectors, MVA vectors, isomaxmet, yeast protei ns, prime-boost approaches using DNA–protein, MVA–Adeno, and Adeno–Adeno. In addition, several of these therapeutic vaccines have been administered to HCV infected individuals. Patients have subsequently been treated with pegylated IFN and RBV. In the future therapeutic vaccines can also be combined with DAA drugs, which will hopefully improve treatment outcome further, particularly in difficult to treat patients.

Mutations within and/or outside immunological epitopes towards which the vaccines are directed may appear after therapeutic vaccinations and new resistant viral strains may emerge. If this happens therapeutic vaccines, being immune modulators via inducing antiviral T cell responses, will need other treatments such as DAs for shutting down the HCV propagation, and IFN and RBV for their antiviral and immune modulatory activity.

2.2. TLR ligands and blocking of PD-1

The first line of defense against invading pathogens (bacteria, viruses, fungi, parasites) is the innate immune system. So-called pattern recognition receptors (PRRs) that recognize pathogen associated molecular patterns (PAMPs) are an important component of the innate immune system (Matzinger, 2002). The most prominent members of the PRRs are the Toll-like receptors (TLRs), which can be divided into two groups with regard to their expression (Kawai et al., 2005).

![Fig. 3. Example of known resistance mutations to DAs located within known CTL epitopes within HCV. Resistance mutations occurring within known human CTL epitopes are shown in red.](image-url)
and Akira, 2011). TLR3, -7, -8 and -9 are located on endosomes and detect nucleic acids like single- (TLR7, -8) and double-stranded RNA (TLR3) and CpG-containing DNA (TLR9), while TLR1, -2, -4, -5 and -6 are expressed on the cell surface where they recognize mainly cell wall components from bacteria and fungi (Kawai and Akira, 2011). The TLRs are composed of an ectodomain that binds the PAMP and a cytoplasmic TIR domain responsible for downstream signalling (Kawai and Akira, 2011). Binding of the respective ligand leads to receptor dimerization and recruitment of a cascade of adaptor proteins like myeloid differentiation primary-response protein 88 (MyD88), TIR domain-containing adaptor protein (TIRAP), TIR domain–containing adaptor protein inducing IFN-β (TRIF) and TRIF–related adaptor molecule (TRAM). MyD88 is used by almost all TLRs except TLR3, which induces IFN-β via TRIF and the IFN-γ-regulated factor 3 (IRF3). The MyD88–dependent pathway leads to the release of nuclear factor –κB (NF–κB) from its inhibitor using a cascade of signalling molecules. NF–κB translocates into the nucleus and induces expression of inflammatory cytokines e.g. IL-6, IL-10, IL-12 and TNF–α (Kawai and Akira, 2011).

In HCV infection the viral RNA is recognized by retinoic acid inducible gene I (RIG-I) and TLR3, which leads to the activation of IRF3 and NF–κB, mediating an antiviral state (Seth et al., 2005). In order to establish a chronic infection, HCV interferes directly with these defense mechanisms of the innate immune system (Gale and Foy, 2005). The HCV NS3/4A encodes a serine protease that cleaves TRIF and CARDIF, disrupting the signalling cascade downstream of TLR3 and RIG-I (Li et al., 2005; Meylan et al., 2005). Finally, a recent study tested the effect of blocking PD–1 in patients with chronic HCV with a limited effect (Gardiner et al., 2013).

Due to the significance of TLRs for the innate immune response towards viruses, the use of TLR ligands to stimulate/enhance the anti-viral response is an interesting approach.

2.3. Neutralizing antibodies

An obvious way to modulate the host response is to increase the levels of neutralizing antibodies (NABs). This can be achieved either by the addition of exogenous neutralizing antibodies, such as a cocktail of HCV envelope (E) 1 and/or E2–specific NABs. An alternative way is to induce these trough vaccination, as has been done in both chimpanzees and in humans with some success. It is fully conceivable that an increase in NABS may improve on the effect of DAAs by simply blocking the ability of the remaining virus to infect new cells. However, limitations are that the HCV E1/E2 proteins are those with the highest genetic variability and that HCV may spread directly from cell–to-cell and not being accessible to NABs.

2.4. T cell therapy

Virus–specific CTLs have a key role in the elimination of virus–infected cells and this includes HCV. However due to the potency to induce chronicity of the HCV, patients infected with HCV ultimately demonstrate a substantial loss of T cell function as a result of T cell exhaustion. The loss of the ability to recognize HCV viral peptides is first shown as lack of interleukin (IL)–2 production, proliferative capacity, and ex vivo killing, which is followed by an impaired ability to secrete IFN–γ at a final stage of CD8+ T cell exhaustion (Kim and Ahmed, 2010; Klenerman and Thimme, 2012). T cell exhaustion may ultimately end with subsequent deletion of antigen–specific T cells, thus prevents optimal control of chronic HCV infections (Wherry, 2011). Given that CD8+ T cells are key immunologic players in HCV infection but ultimately fail in its ability to eradicate the virus, a new “T cell redirection” approach has recently been proposed to produce functional HCV–specific effector T cells, that can be regarded as a passive therapeutic vaccination. This approach relies on T cell receptor (TCR) transfer, by which the antigen specificity can be transferred from one T cell to another by gene transfer. TCR transfer is a cornerstone that has led to generation of a number of TCR transgenic and retrogenic animal models (Bettini et al., 2012; Chen et al., 2001, 2005). Lately it has opened up new possibilities to treat tumour patients (Restifo et al., 2012).

In the case of HCV, cloned αβ TCR from CD8+ T cells with specificity to NS3 (Callender et al., 2006) as well as NS5 (Pasetto et al., 2012a) have been identified and transferred recently into new T cells from HCV–negative human individuals. The new CD4+ and CD8+ T cells from HCV–negative individuals confer anti-HCV reactivity and recognized HCV peptide-loaded target cells and HCV–positive hepatocellular carcinoma cells. To date, TCRs with specificity to the NS3 1073–1081, NS3 1406–1415 or NS5A 1992–2000 have been successfully identified and restored on new human T cells. The HCV NS3 TCR transfer may redirect T cells from healthy as well as chronic HCV patients (Callender et al., 2006; Pasetto et al., 2012b; Zhang et al., 2010) to recognize HCV target cells with a polyfunctional response (production of IFN–γ, TNF–α, IL–2 and CD107a expression). Moreover, the NS3 1073 and NS5A 1992–2000 TCR–redirected T cells demonstrated an effective elimination of HCV replication in HLA–A2+ Huh–7 hepatocellular carcinoma cells (HCV–replicon cells) that persistently replicating a subgenomic HCV RNA (Pasetto et al., 2012a).

Interestingly, whilst the NS3 1073 TCR resembles a high avidity TCR – capable of instructing T cells to become polyfunctional effector cells to kill HCV+ hepatocytes in a cytolytic and HLA–A2–restricted manner; the NS5A 1992 TCR instead shows features that resembles a low/medium avidity TCR. It was found that redirected T cells by each of the TCR exert an effective antiviral effect on HCV replicon cells, however the elimination of HCV RNA+ cells appears to occur in a non–cytolytic fashion after incubation with the NS5A 1992 TCR–redirected T cells (Fig. 4). Unlike the NS3 1073 TCR (gives rise to high avidity, polyfunctional, cytolytic CTL) identified and provided with the same approach, the low/medium avidity NS5A 1992 TCR appears to give rise to mostly non–cytolytic CTLs dominating by IFN–γ or TNF–α producing cells. Although high avidity TCRs like NS3 1073 may be more effective in eliminating HCV+ target cells, there is benefits with non–cytolytic T cells generated with for instance the NS5A 1992, as it may spare the host from unwanted tissue damage by having less hepatotoxic potentials (Pasetto et al., 2012a). Adding NSSAT cells might be an advantage when reconstituting a multi–specific antiviral T cell response in vivo to avoid the potential overkilling of hepatocytes, however the ultimate answer to which of the T cells are more beneficial remained to be evaluated in vivo.

The new HCV TCR–redirected T cells represent some new tools for immunological studies of HCV and may have some clinical potential to help HCV patient groups that are prone to failure of standard therapy. They may also serve in new therapeutic combinations with for instance antiviral drugs to meet the potential challenges from resistant virus strains (Fig. 4).

3. Immune modulation in the clinic

3.1. Vaccines

An increasing number of therapeutic vaccine trials have been performed although none have so far provided any cure for HCV. On the other hand the performed trials have shown some success, such as reducing the viral load, activated HCV–specific T cell responses, and last but not least, proven to be safe, tolerable and associated with less adverse events compared to SOC therapy with peg–IFN plus RBV+/– a first generation PI. An interesting approach being tested in a larger number of therapeutic vaccine trials is the combination of vaccine with pegylated IFN and RBV. A brief account of the performed and ongoing therapeutic vaccine trails are described
below. Intercell AG (Vienna, Austria) has evaluated a peptide-based vaccine (IC41) alone or with an adjuvant (poly-L-arginine) in phase II clinical trials (Firbas et al., 2006; Klade et al., 2008; Wedemeyer et al., 2009). In general this vaccine composition was safe and tolerable, but induced only modest decrease in viral load, and no significant correlation between HCV viral load decline and activation of HCV-specific T cell responses was seen. Moreover, IC41 immunization as an add-on to pegylated IFN and RBV treatment in chronic HCV patients did not improve cure rates (Wedemeyer et al., 2009). Another approach utilized by Transgene S.A. (Illkirch-Graffenstaden, France), is an attenuated non-replicative vaccinia virus Ankara (MVA) strain as a vector for delivery of HCV genes to the infected host. In clinical phase I and II trials the TG4040 vaccine demonstrated a good safety profile, induction of IFN-γ producing T cell responses, and when given in combination with pegylated interferon and ribavirin an early rapid virological response was seen in HCV infected patients (Habersetzer et al., 2011; Wedemeyer et al., 2011, 2012b). In the same line, Okairos (Rome, Italy) have generated Adenovirus vectors based on rare serotypes, utilizing a prime-boost approach where human adenovirus 6 (Ad6) and chimpanzee adenovirus 3 (ChAd3) are expressing HCV genes. The vaccine composition primed potent T cell responses in healthy volunteers. The immune response targeted several HCV antigens and were still detectable one year after last vaccination (Barnes et al., 2012). This vaccine still needs to show proof of antiviral activity in chronic HCV infected individuals or protective immunity as a preventive HCV vaccine. Others have utilized plasmid DNA vaccines for treatment of chronic HCV infections. ChronTech Pharma AB (Huddinge, Sweden) has developed a DNA-based vaccine (ChronVac-C) delivered in combination with in vivo electroporation. Results from a phase I/IIa clinical trial showed that the ChronVac-C vaccine was safe and tolerable, induced HCV-specific T cell responses, and was associated with rapid reductions in viral load.

Fig. 4. T cell receptor redirected T cells can eliminate HCV RNA+ hepatocytes. The avidity of the chosen TCR (low or high) may also determine the fate of the target hepatocyte, as it may guide the CTL function towards the cytolytic or the non-cytolytic mechanisms.

Fig. 5. Description of the beneficial effect of combining the HCV-specific T cell response with DAA-based therapy.
cell responses and transiently reduced the viral load (Salberg et al., 2009; Weiland et al., 2013). Interestingly, patients first given the DNA vaccine, who thereafter started pegylated IFN and RBV treatment had an improved cure rate (Weiland et al., 2013). Similarly, GlobImmune (Louisville, Colorado) has developed a yeast cell, inactivated Saccharomyces cerevisiae, expressing recombinant HCV antigens (GI-5005). The GI-5005 vaccine showed a tolerable profile, and given together with pegylated IFN and RBV it resulted in an early virological response (Haberszetler et al., 2009). In summary, several therapeutic vaccines have shown promising results in phase I and II clinical trials although so far none of them could cure HCV infection.

3.2. TLR agonists

TLR agonists have been considered for treatment of HCV due to their potent effect on inducing a local IFN response. So far only a few have been evaluated in the clinic. A disadvantage is that the effect often is systemic with an immune activation also outside the liver. The following section will summarize the current experience.

The TLR7/8 ligand Resiquimod showed little effect in combination with severe side effects at a dose of 0.01–0.02 mg/kg in two clinical phase IIa studies with an oral administration twice weekly for four weeks (Pockros et al., 2007). The treatment seemed to induce IFNs from pDCs via TLR7 with immediate, but transient, decreases in HCV RNA levels (Pockros et al., 2007).

The TLR7 agonist isatoribine has been found to transiently reduce HCV RNA levels in plasma (Hormans et al., 2005). Also a prodrug of isatoribine termed ANA975 was converted to isatoribine in humans (Xiang et al., 2007). Several TLR7 agonists are currently in clinical testing, for example GS-9620, but not data has yet been presented.

The TLR9 agonist CPG10101 has been tested in HCV infected patients administered subcutaneously twice weekly for four weeks. This study showed that CPG10101 induced an immune activation with transient effects on HCV replication (McHutchison et al., 2007).

The TLR9 agonist IMO-2125 has been tested in HCV infected null responders and did show transient decreases in HCV RNA levels of up to 3.5log (McHutchison et al., 2010). A phase IIb study was terminated early (EASL, 2010).

Overall, the TLR agonists have shown promising transient effects in HCV infected patients and may well be evaluated in combination with the IFN free DAA combinations.

4. Combination of DAAs/antivirals and immune modulation in the clinic

4.1. Vaccines, antibodies and TLR agonists with IFN/RBV

The strongest evidence that immune modulation is a key component of DAA-based therapies is the difficulty to remove ribavirin from the treatment regimen with retained treatment results. This is particularly true for patients infected by gt1a. Thus, the host immune response seems to be essential for elimination of HCV in the forthcoming era of highly active DAA-based therapies.

Having acknowledged that the host immune response is needed for a treatment to be effective a question arises – which way is the best way and most attractive to achieve modulation of the host response? The most well proven immune modulator is of IFNα. However, a major disadvantage with IFNα treatment, however, are the many and often severe side effects. This is a major reason for eliminating IFNα from the treatment of HCV infected individuals.

Concerning therapeutic vaccines and their utilization in combination with HCV antiviral drugs, the only treatment reported so far is such a combination with IFN/RBV. In this setting at least three different vaccines have been tested, a DNA vaccine delivered by in vivo electroporation (Salberg et al., 2009; Weiland et al., 2013), a modified vaccinia virus Ankara strain (MVA) (Wedemeyer et al., 2012b), and yeast cells carrying HCV proteins (Haberszetler et al., 2009). These studies have all supported that the addition of therapeutic vaccination can improve either the early viral kinetics and/or the sustained viral response. Hence, they all support the concept of adding a therapeutic vaccination to the IFN therapy. Still lacking, however, are studies including such a combination with DAAs. A first ongoing controlled study in 32 patients will test treatment with, or without, DNA-based vaccination followed by 12 weeks of IFN/RBV treatment. After this a first generation protease inhibitor will be added for the patients who has not achieved a more than 2 log drop in HCV RNA levels week 12 (Weiland et al., personal communication). This study will tell us whether the combination of a therapeutic vaccine, IFN/RBV and a DAA can be utilized and overcome treatment failures in difficult to treat patients with chronic HCV infection.

5. Concluding remarks

5.1. Is there a role for immune modulation in future HCV therapy?

Considering the potentially high effectivity of the coming DAAs in cutting down the HCV replication, is there still a need for immune modulating compounds? There is obviously no clear cut answer to this question, but the following factors should be considered. In real-life therapeutic situations outside clinical trials, treatment results are in many cases much less effective. In particular when considering three to six months of oral therapies where the patients must take a dosing twice or thrice per day, the regimens are totally dependent on patient compliance. With this background an immune therapy administered on a bi-weekly to monthly basis that will help boost an immune response and an endogenous control of the infection might be very helpful. With the new DAAs which effectively suppress the viral replication, the HCV induced “immune blockade” of the host response both directly and indirectly by the mere presence of HCV antigens in the circulation can be broken. Subsequently the endogenous T cell response can be effectively activated with an immune modulating therapy such as a therapeutic vaccination. With this help the host activated immune response can eliminate the remaining infected hepatocytes and finally achieve clearance of the chronic HCV infection (Fig. 5).

References


