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THE INTERNATIONAL ARABIC JOURNAL OF ANTIMICROBIAL AGENTS

2014
Vol. 4 No. 1:1
doi: 10.3823/743

Immune-based strategies for treatment and prevention of hepatitis C virus infection

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Abstract

According to the current epidemiological studies, 3% of the world population is infected with hepatitis C virus (HCV). The gold standard of therapy, at this time, is neither effective in a high percentage of patients, nor with all genotypes .Furthermore, it is associated with significant side effects and high cost. Therefore, new therapeutic strategies have been investigated, or in the early process of development. These include: direct acting antiviral agents (DAAs) and immune-based therapy. Four DAA molecules have been already approved by the FDA. Immune based therapy aims at augmenting host immunity, thus prevention of infection, and clearance of the virus in subsequent re-infection. Boosting T cell responses and activating humoral immune reaction have been targeted in the development of novel fighting tools. The most intensively studied immune-therapeutic strategies are: **1)** vaccines; either therapeutic or prophylactic, **2)** dendritic cells (DCs) immunotherapy, **3)** antagonists of T cell inhibitory factors, **4)** anti-HCV neutralizing antibodies (nAbs), **5)** antagonists for Toll-like receptors (TLRs), and **6)** caspase inhibitors.

Globally, 170 million people are chronically infected with HCV, [1]. Furthermore, it is estimated that the world reservoir of HCV infected individuals is in a steady increase, which is not limited to developing countries [2].

Despite a long battle within the field of control and treatment, HCV infection remains to be a serious health problem. That is due to the belatedly complications of life-threatening liver diseases, including

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livercirrhosis and hepatocellular carcinoma, [3]. HCV has also been incriminated among the most important causative factors for type II mixed cryoglobulinemia (MC) [4].

Current gold-standard therapy for HCV infection is weekly injections of pegylated interferon combined with daily oral ribavirin. In addition to being expensive, this therapy is fraught with significant side effects. Response varies with different genotypes. A sustained virologic response (SVR) of 40- 50% is achieved with genotype 1. Higher SVR rates are achieved in patients infected with HCV genotypes 2, 3, 5, and 6 (up to about 80%, and better for genotype 2 than for genotypes 3, 5, and 6, and intermediate SVR rates were achieved in those with HCV genotype 4 [5]. Given these results, exploration of new therapeutic strategies with higher efficacy and less side-effect is urgently needed.

In recent years, researchers have focused their efforts on developing novel molecules with antiviral effect, as well as emphasizing immune-based therapy. Four molecules with anti- HCV effect are now available; boceprevir, telaprevir, sofosbuvir and simeprevir [6-7]. In spite of the capability of these molecules to increase SVR rates, it is even far more costly, and is associated with potential drug-drug interactions and substantial side effects.

Immune-based strategies can represent an intriguing alternative as therapeutic as well as prophylactic approach. When HCV infection occurs, nearly 15-30% of acutely infected individuals can spontaneously eradicate the virus [8]. In addition, significant levels of innate immunity to HCV have been reported in studies of the chimpanzee model [9] and in studies of reinfections in intravenous drug users [10]. These findings have highlighted the fundamental role of innate immune response in the control and resolution of HCV infection. Such pessimistic view has also been moderated by significant advances in our understanding of the immunological correlates and mechanisms underlying the successful control of viral infection [11]. All these findings have led to a conclusion that prevention and/or cure of HCV is possible if similar immune responses can be elicited. T cell responses have been targeted in the development of novel combating tools, and therapeutic strategies for activating humoral immune responses have also been under development.

Immunological strategies for prevention/treatment of chronic HCV

Chronic HCV infection cases resistant to conventional therapies, or those who are relapsers might be treated by immunotherapy-based strategies. These strategies may also endow with a safe, effective and affordable prophylactic approach, providing the best long-term hope for controlling the global epidemic, thereby decreasing the burden on healthcare systems.

In this review, we will discuss immunological strategies that could be used as therapeutic and/or prophylactic agents for HCV infections. These include HCV vaccines, DCs immunotherapy, antagonists of T cell inhibitory factors, anti-HCV nAbs, cytokines and chemokines, agonists for TLRs, and caspase inhibitors, **figure (1)**.

Vaccines

The development of vaccines against HCV is hampered by several challenges. First of all, is the genetic heterogeneity which is a hallmark of HCV as an RNA virus [13]. Moreover, there are technical limitations in the study of HCV, mainly as regards the limited availability of convenient small animal

Received on 28 April 2014; accepted after revision on 24 May 2014

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Vol. 4 No. 1:1
doi: 10.3823/743

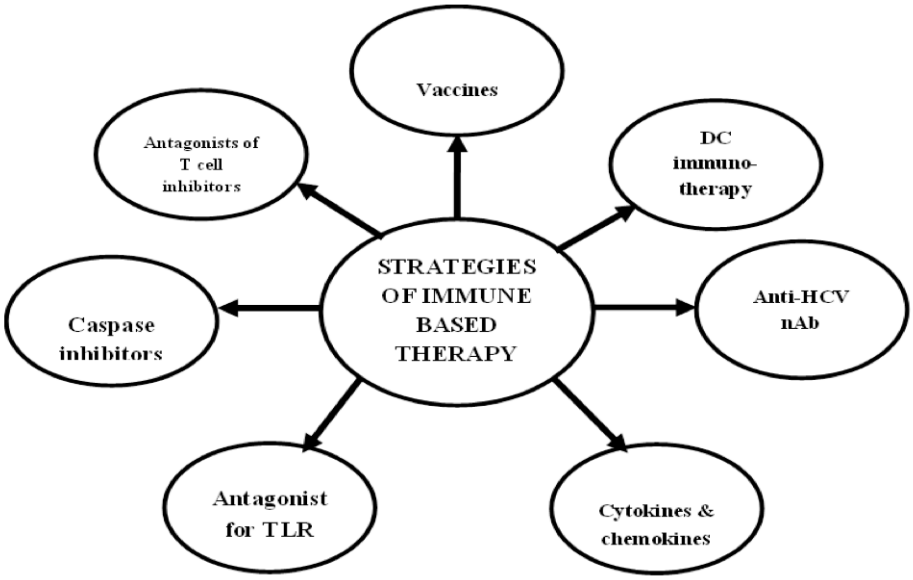

Figure 1. Strategies of immune-based therapy [12].

models other than the chimpanzee, mimicking HCV infection in humans. Another challenge was the difficulty of growing the virus in cell culture, a problem which has been recently overcome [14].

A successful HCV vaccine should address viral heterogeneity, cover the various genotypes and quasi-species of HCV, and must incorporate epitopes from HCV structural proteins in their correct three-dimensional conformations, to induce the production of high titers of broad nAbs. It must also incorporate HCV-specific T-cell epitopes from HCV nonstructural proteins, to elicit strong cellular responses [15-17].

Both prophylactic and therapeutic vaccine candidates have been developed. The general principals pertaining to the different vaccination strategies include: recombinant protein vaccines, including the recombinant yeast-based vaccine candidates, virus-like particles vaccines, peptides vaccines, DNA vaccines, viral vector vaccines, and DCs- based vaccines. DC-based vaccines will be discussed later. The most recent achievement in the development of vaccines in the other categories are shown in **table 1**.

Candidate vaccination approaches which are consider the most recent, the most considered and, perhaps, the most promising are: the recombinant protein vaccines [20] the adenovirus-based vaccine, [29] virus-like particles- based [21] and the DNA vaccines candidates [25].

As the resolution of HCV infection is frequently associated with high quality cellular immunity and the production of nAbs, conceivably, combining the approaches that p rime both humoral and cellular immunity would protect more efficiently against HCV challenge. It would be very interesting to investigate, in a prime-boost regimen, a combination of vaccines; the first to target cellular immune responses, and the second to target nAbs formation. The first could be the adenovirus-based vaccine [29] while the second could be a virus like particles-based vaccine [21]. Although this concept remains to be tested in suitable animal models and/or clinical trials, it should be possible to develop vaccines with at least partial efficacy against HCV-induced chronic liver disease [21].

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Table 1. The progress in the development of preventive and therapeutic vaccines for hepatitis C virus.

Strategy	Vaccine	Manufacturer	Aim	Challenge inoculum	Study model (Phase)	Outcome	Ref
Recombinant protein	Chiron Corp HCVE1/E2 vaccine	NIAID Novartis	Prophylactic	Recombinant E1 and E2 proteins with MF59 adjuvant.	Human Phase I Phase II planned 2012-2013	Neutralizing antibodies to hepatitis C	18, 19
Recombinant protein				Recombinant gpE1/gpE2 from a single strain (HCV1 of genotype 1a).	Human 2013	Minority of vaccinees, elicited broad cross-neutralization against all HCV genotypes	20
Virus like particles (VLP)	Bivalent HBV/ HCV vaccine		Prophylactic	Chimeric HBV–HCV E1-S and E2-S proteins.	Rabbit 2013	Strong specific Ab response against the HCV and HBV envelope proteins	21
Virus like particles (VLP)	HCV-specific plasmo-retroVLPs		Prophylactic	Plasmid DNA forming VLPs pseudotyped with HCV E1 and E2 envelope glycoproteins.	Mice 2013	Induce broad cellular and humoral immune responses.	22
Peptide	IC41	Intercell AG	Therapeutic	HCV peptides with polyarginine.	Human Phase II 2012	Safe and well tolerated. Specific T cell responses. Weak viral load reduction.	23, 24
DNA vaccines	(Chron Vac-c)	Inovio	Therapeutic	DNA based vaccine CHRONVAC- C® in collaboration with elecroporation, followed by Standard of care therapy.	Human Phase II 2013	Excellent safety profile. Elimination/ reduction of HCV viral load.	25
DNA vaccines	INO 800 HCV	Inovio	Therapeutic	Synthetic multi-antigen DNA vaccine covering HCV genotypes 1a and 1b and targeting the antigens NS3/4A.	Phase I/IIa (4Q 2013)		26

Received on 28 April 2014; accepted after revision on 24 May 2014

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Viral vector	TG4040	Transgene	Therapeutic	MVA vector expressing HCV antigens including NS3, NS4, and NS5B, followed by SOC	Phase II	Well tolerated, decline in HCV viral load and increased early response rates of SOC.	27, 28
Viral vector	Adenovirus-based vaccine		Prophylactic	HAd6 and ChAdCh3, expressing nonstructural proteins of HCV	Human Phase I	Safe and well tolerated. Highly immunogenic response, with the induction of robust, cross-reactive and sustained CD4+ and CD8+ T cell-mediated responses.	29

Dendritic cell immunotherapy

In the last decade, considerable progress has been made in the field of DC biology [30]. Furthermore, promising results have been published for immunization against the human immunodeficiency virus, [31] and Epstein-Barr virus (32). On April, 2010 the DC vaccine PROVENG was approved by the FDA to treat prostate cancer [33]. Consequently, a scientific focus has been brought to the critical effect of DCs as an important element of immune-based strategies for both treatment of, and prophylaxis against HCV.

A therapeutic DC vaccine, in addition to the potent capability of T cells priming, is supposed to bypass the suboptimal microenvironment and/or diminished DC function in chronic hepatitis C patients *in vivo*. DC-based vaccines are also expected to be encouraging as prophylactic for high-risk populations by induction of both CD8+ T cells and CD4+ Th cells [34].

DC-based vaccine development has used a number of proteins as antigens either separate or in various combos. They are HCV proteins both structural and non-structural, as core, [35] NS3, [36] NS4a, [37] NS5, [38] and NS5b [39]. HCV-like particles [40] and the most recent is HCV pseudoparticles (HCVpp). [41]

DC-based vaccine development has also used a strategy which is based upon *ex vivo* stimulated and matured DC loaded with HCV specific antigens, which are then delivered into host [42].

Starting from 2001 there is a large body of literature involving the use of various vehicles for loading DCs with various HCV antigens for the purpose of developing potent therapeutic or prophylactic vaccine candidate, or of developing model systems to study HCV-DCs interaction, **figure 2**. They are; adenoviral vector, [43] recombinant adeno-associated virus (rAAV), [37] anthrax toxin fusion protein, [44] vaccinia virus, [45] lentivirus, [46] pesti virus, [47] microbeads vectors, [48] a protein delivery based on a short amphipathic peptide carrier; Pep1 [49], extra domain A from fibronectin (EDA), [50] and the lipid Pam2Cys [51].

Recently, dendritic cell (DC) -based vaccines against HCV has been created. Some of DCs vaccination strategies were even developed into clinical trials targeting chronic HCV subjects [52-54].

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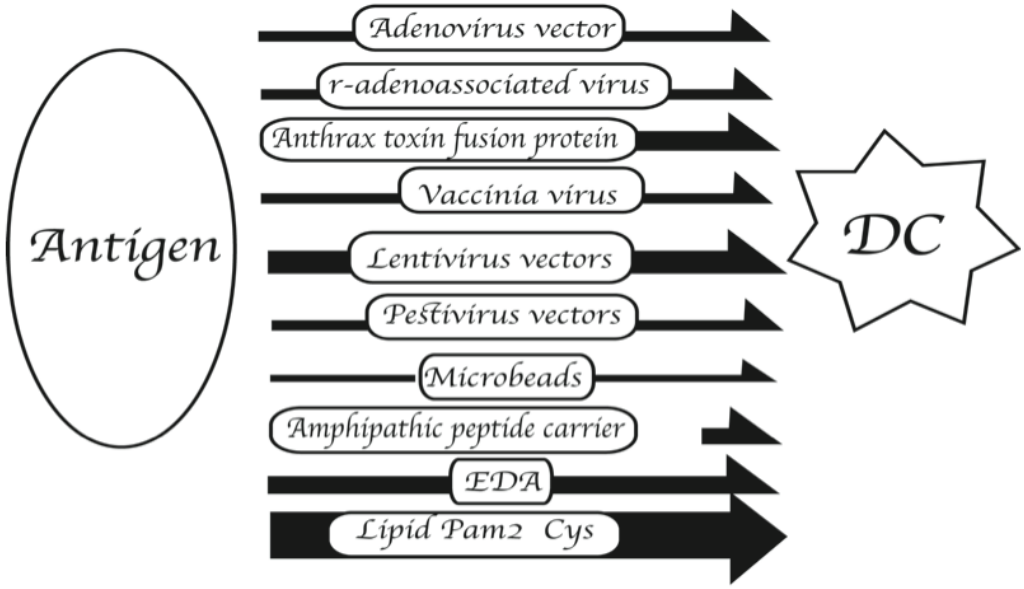

Figure 2. Antigen loading on DCs, [12].Vehicles for loading DCs with HCV antigens, including: adenoviral vector, rAAV, anthrax toxin fusion protein, vaccinia virus, lentivirus, pesti virus, microbeads vectors, a protein delivery based on a short amphipathic peptide carrier; Pep1, EDA, and the lipid Pam2Cys.

Antagonists of T cell inhibitory factors

T cells are exhausted and overexpress inhibitory molecules in chronic HCV infection. These include cytotoxic T lymphocyte antigen-4 (CTLA-4), [55] programmed death-1 (PD-1) inhibitory receptor, [56] B7 family member B7-H4, [57] T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), [58] and lymphocyte activation gene-3 (LAG-3) [59]. Interaction of these molecules with their cognate ligands on various cells types result in reduced T cell propagation and function, [60] together with tolerance to the antigens' exposure [61]. Indeed, one of the primary goals of immune- therapy is the functional reversal, or reactivation, of tolerized T cells in settings of chronic antigen exposure. Novel HCV treatments based on blockade of these inhibitory molecules are being investigated.

Tremelimumab is a fully human IgG2 monoclonal antibodies (mAb) directed against CTLA-4. Clinical trials for HCV disease are still underway [62].

Blockade of PD-1 signaling can restore function to exhausted virus-specific CD8 T cells. [63] MDX-1106, is a fully human antibody also known as ONO-4538, and CT-011. It interacts with PD-1 receptor, and is being developed as treatment for cancer disease and for therapy of chronic HCV infection. [64] Clinical studies to evaluate the use of CT-011 in HCV disease have been initiated [65].

Co-expression of PD-1 and CTLA-4 was observed in liver-infiltrating lymphocytes, but not in peripheral blood lymphocytes, suggesting the phenotypic differences of virus-specific CD8+ T cells in different *in vivo* compartments. PD-1 and CTLA-4 expressing HCV-specific T cells were profoundly dysfunctional [66]. It is important and possible to consider the combination of PD-1 and CTLA-4 blockade for immunotherapy to enhance antiviral immune responses. [67]. Mbs interfering with the PD-1 and CTLA-4 pathways, [68] are currently being studied in clinical trials.

Silencing B7-H4 by small interfering RNA (siRNA) in activated hepatic stellate cells (HSC) restored the ability of T cells to proliferate, differentiate, and regain effector recall responses. Furthermore, addi-

Received on 28 April 2014; accepted after revision on 24 May 2014

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tion of exogenous interleukin (IL)-2 reversed the T cell anergy induced by activated HSC [69].

Recent studies have shown that higher expression levels of dual Tim-3 and PD-1 has been reported to correlate with impaired Th1/Tc1 cytokine secretion and diminished cytotoxic potential. Their combined blockade of T cell immunoglobulin and mucin domain–containing molecule 3 (Tim-3) represents a potential target for novel immunotherapeutic approaches [70].

Studies have shown that inhibition of LAG-3 through Ab blockade can reverse the tolerogenic state, and partially restore effector function of immune cells [71].

Anti-HCV neutralizing antibodies

Recent reports of human monoclonal antibodies (nAbs) neutralizing genetically diverse HCV isolates and protecting against heterologous HCV quasispecies challenge have validated the concept of the use of neutralizing antibodies to prevent HCV infection [72].

Current understanding of the nAbs response raised against HCV suggests that E2 is the major target, and that multiple epitopes within E2 may be targeted by both linear -and conformation-dependent antibodies. Predominantly, these neutralization epitopes overlap with CD81-binding sites, [73] and clearly demonstrate a role in inhibition of entry. A number of anti-receptor Abs targeting SR-BI, has been reported [74].

Given the potential antiviral effect of the antibodies, HCV has evolved multiple mechanisms for protection from antibody binding. These include glycosylation of receptor, and lipid shielding [75]. More recently, HCV has been found capable of direct cell-to-cell transmission, which is largely resistant to antibody neutralization [76]. Finally, the high mutation rate of HCV, constitutes a significant hurdle for nAbs development [77]. All these problems may be counter-balanced by selecting nAbs which target conserved and more accessible areas of viral particles, and/or by using mixtures of nAbs which target various key epitopes. In fact, it has been demonstrated that combination therapy with nAb cocktails prevents escape variants for many viruses, including influenza, and others [78].

MBL-HCV1 is one of the monoclonal antibodies against the viral antigens. A phase I open-labelled, dose escalation study was performed in healthy adult volunteers. MBL-HCV1 was well-tolerated without any seriously adverse effects. Based on the favourable safety, tolerability and pharmacokinetics data, a phase II study of MBL-HCV1 in chronically infected HCV patients undergoing liver transplantation has been planned [79].

Another therapeutic Ab which targets host antigens is bavituximab. It is the first in a new class that targets and, preferentially binds, to phosphatidylserine which is exposed on the surface of certain atypical animal cells, including cells infected with HCV [80]. Among clinical trials using bavituximab, it is currently being evaluated in randomized phase II clinical trial for therapy of chronic HCV infection and for HIV/HCV co-infection [81]. The available results are promising.

Cytokines and chemokines

Cytokines play an important role in the defense against viral infections. In HCV infection, cytokines are involved in either viral control or liver damage [82].

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Interferons-alpha (IFNs-α) is the only cytokine currently used in the treatment of chronic viral hepatitis [83]. On the other hand, ribavirin, a wide-spectrum antiviral agent used in combination therapy for hepatitis C, has immunomodulating effects that induce type 1 cytokine production [84].

In addition to IFNs-α, IFN-γ was shown to suppress HCV replication. A comparative analysis demonstrated that IFN-γ exerted a more prolonged antiviral effect than IFN-α and IFN-β in the HCV RNA replicon system [85].

In addition, cytokines involved in the regulation of cytokine networks could be combined with cytokine therapies to enhance the efficacy. In this context, recombinant IL-2 was tested in patients with chronic HCV infection. However, IL-12 therapy was poorly tolerated and showed low efficacy as regards SVR [86].

Other anti-inflammatory cytokines such as IL-10 has also been attempted to reduce intrahepatic inflammation severity. [87]. However, such therapies remain experimental, and their effectiveness is unclear.

From a theoretical standpoint Tc1-associated chemokine receptors may represent an interesting therapeutic target in the development of drugs for patients with chronic hepatitis unresponsive to antiviral agents. Their aim being a reduction of liver inflammation and progression to fibrosis by blocking inflammatory cell migration into the liver [88].

Another important cytokine is the IFN-lambda, which is expected to become the next cytokine storm. It upregulates IFN-stimulated genes similar to IFN-alpha and -beta, but via a different receptor. There is also evidence that IFN-lambda affects the adaptive immune response [89]. Clinical studies assessing safety and efficacy in the treatment of HCV with exogenous IFN-lambda3 are of great importance. Results suggest that IFN-lambda 3 treatment inhibits HCV replication and is associated with a limited side effect profile. However, hepatotoxicity has been described [90].

Pegylated interferon lambda was also evaluated in combination with DAAs [91]. This international Phase 2b trial enrolling patients with HCV genotype 1 as well as a Japanese study enrolling patients with HCV genotype 1b infection [92], yielded extremely encouraging results. Both studies recommended further investigation in a larger population.

Agonists for TLRs

Therapeutic applications of TLR to date have used two strategies. One involves synthetic versions of natural TLR ligands with optimized pharmacologic properties, and the other encompasses small molecule agonists derived from drug screening effort, (Amer F. 9th Arab Conference for Antimicrobial Agents. Beirut, Lebanon, 2012).

TLR agonists are undergoing intense drug development in different fields including chronic viral infections, oncology and vaccines. Agonists for TLR3, TLR7, TLR8 and TLR9 have shown promise as treatments for infectious diseases, especially viral infections. [93] A current knowledge is available on the use of many TLR agonists for HCV treatment, **table 2**.

Caspase inhibitors

Increased apoptosis is a hallmark of active hepatitis C infection, and the level of hepatocyte apoptosis correlates with the histologic activity grade. It has been suggested that hepatocytes apoptosis may be a mechanism for viral shedding, so, inhibition of apoptosis could ameliorate HCV [101].

Received on 28 April 2014; accepted after revision on 24 May 2014 This article is available from: www.iajaa.org

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Vol. 4 No. 1:1
doi: 10.3823/743

Table 2. Anti-HCV drugs targeting TLRs R: references

Compound	Target	Company	Status	R Ref.
ANA975	TLR7	Anadys	Phase Ib	[94]
ANA773	TLR7	Anadys	Phase IIa trials.	[95]
CpG-ODN	TLR9		Preclinical	[93]
CpG10101	TLR9	Coley	Phase II (discontinued)	[96]
GS-9620	TLR7	Gilead	Randomized,double-blind phase I. Active, not recruiting participants	[97]
Thymosin α1	TLR9, 2 on DCs & precursor T cells	SciClone	Addition to an IFN-based regimen.	[98]
Resiquimod	TLR7/8	Spirig Pharma Ltd	Double-blind, phase IIa studies.	[99]
IMO-2125	TLR9	Idera	Dose escalation, proof of concept, phase I study.	[100]

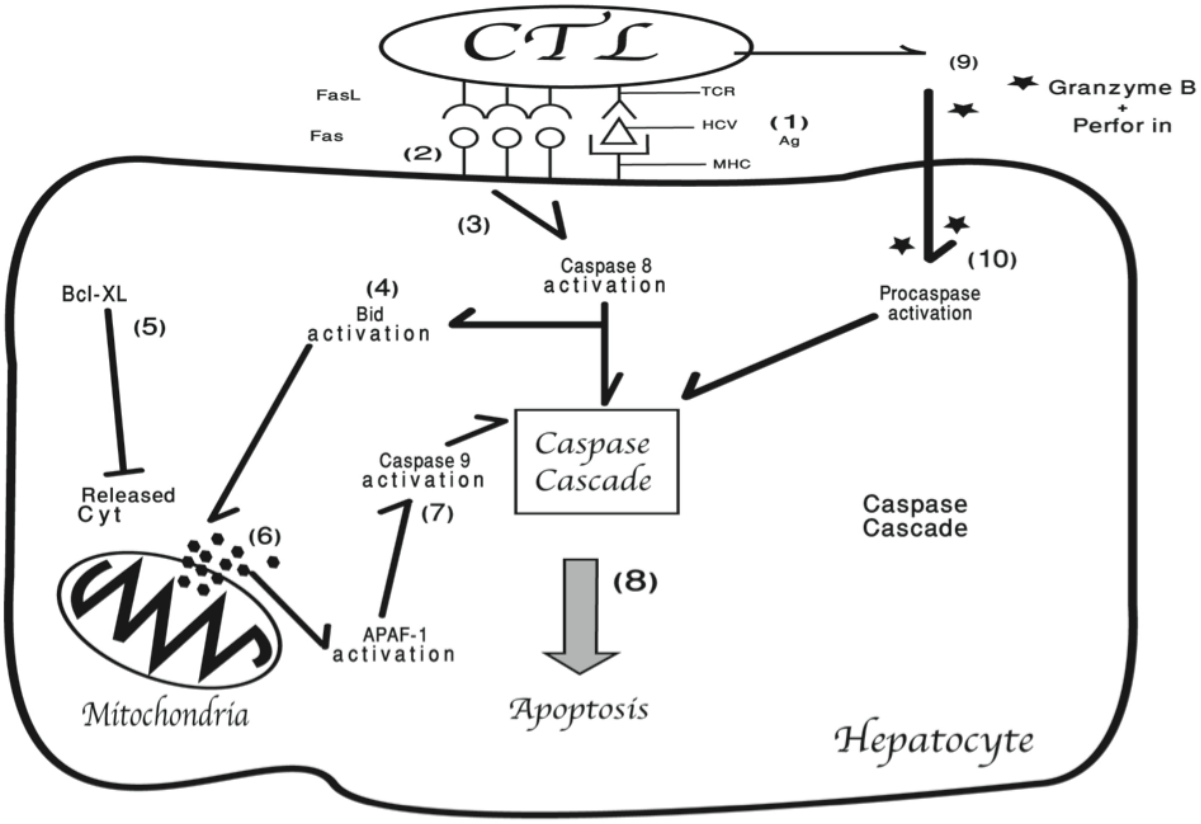

Figure 3. Apoptosis pathways, adapted from Mita, et al [102].

TCR: T cell receptor. HCV: hepatitis C virus, MHC: major histocompatibility complex, Fas: a transmembrane receptor of the tumor necrosis factor (TNF) receptor superfamily. FasL: Fas ligand, Bid: BH3 interacting-domain death agonist Bcl-XL: B-cell lymphoma-extra large. A transmembrane molecule in the mitochondria Cyt: cytochrome. APAF1: apoptotic protease activating factor 1

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Apoptosis is carried out via two pathways; death receptor–mediated pathway, and mitochondrial apoptotic pathway, **figure (3)**. HCV infection has been shown to influence both pathways [102].

The two pathways involve a sequence of reactions; the common end point of both is the activation of the cascade of the intracellular proteolytic enzymes caspases [103]. Since they are essential in cells for apoptosis, caspase inhibition often abrogates apoptosis. Consequently, interest as potential therapeutic targets, has emerged. Other factors were also found to contribute to caspases being an attractive therapeutic target in the setting of HCV. These include; **i)** caspases are activated in human biopsy specimens of chronic HCV patients, **ii)** their activation precedes morphological evidence of cell death, **iii)** about four times more apoptotic cells are detected in HCV-infected liver tissue compared to control, **iv)** caspase inhibitors protect hepatocytes from death signals during HCV infection and organ stress, **v)** apoptotic markers are decreased in HCV patients after treatment with caspase inhibitors, and **vi)** clinically, the ultimate result of caspase inhibition could be an overall reduction in the negative consequences of the host response to the virus infection (i.e., reduction in inflammation, apoptosis and fibrosis). [104 So far, three caspase inhibitors have been tried for treatment of HCV infection; the pan-caspase inhibitor ZVAD-fmk [105], emricasan/IDN-6556 [106], and MitoQ [107]. The first produced decreased mortality in the rat massive hepatectomy model of acute liver failure, while the other two were well tolerated and may halt disease progression to fibrosis or cirrhosis.

Conclusions

Thanks to new advancements in medicine and dedicated scientists, we now have an established body of knowledge regarding how the body reacts and interacts with HCV. We also know about the chain of events from the moment the virus enters the host, and how some infected patient eradicate the virus without medical interference.

The real challenge is how we can utilize this knowledge to find new ways to prevent or cure this disease. As illustrated, this could happen by augmenting the body's cellular immune response, thus providing the means to resist the virus as observed in the 15-30% of infected individuals who eradicated the virus spontaneously.

There are several ways to promote the immune response to such levels, including preventive and corrective vaccines, targeting the DC cells that orchestrate functions of the immune system, finding antagonists of T cells inhibitory factors, anti-HCV nAb, identifying effective cytokines and chemokines, agonists of TLR, and caspase inhibitors.

Many studies are currently underway or being conducted and the results were placed across the effectiveness continuum. While some expressed the need for longer time to have meaningful results, others have shown promising and more positive outcomes. The later have been moved to clinical trials on human subjects.

HCV is a global disease, the morbidity and mortality rates are staggering.The cost of treating this disease is in upward spiral. However, the day of controlling this disease or even eradicating it is not a dream, but a true reality.

Acknowledgement

Thanks are due to Dr. Afdal I. Allam, MD. Medical One Family Medical Group. 8970 Warner Ave, Fountain Valley, CA 92708, USA, for editing the whole manuscript.

Received on 28 April 2014; accepted after revision on 24 May 2014

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Vol. 4 No. 1:1
doi: 10.3823/743

Thanks are also due for Noha Hammad, assistant lecturer, Medical microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt for help in developing figures included in this manuscript.

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Received on 28 April 2014; accepted after revision on 24 May 2014 **This article is available from:** www.iajaa.org

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