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**Progress in the development of vaccines for hepatitis C virus infection**

Ghasemi F *et al.* Preventive and therapeutic vaccines for HCV infection

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# Abstract

The hepatitis C virus (HCV), first described in 1989, is now a leading cause of liver cirrhosis and hepatocellular carcinoma. With more than 170 million people infected globally, this virus is a major public health issue. The current standard therapy is based on interferon in combination with ribavirin. This costly therapy often fails to completely clear the infection and is associated with adverse side effects. Recent anti-HCV therapies are interferon-free direct-acting antiviral (DAA) regimens for HCV such as Simeprevir, Sofosbuvir and Ledipasvir which have effects on non-structural (NS) proteins. DAA regimens have several advantages such as specifically targeting HCV viral replication, accompanied by very high SVR rates and lower side effects such as flu-like syndrome.These facts plus the fact that most HCV cases progress to chronic infection suggest the potential need for an efficient HCV vaccine. Different innovative methods, including methods based on peptide, recombinant protein, DNA, vector-based, and virus-like particles, have been introduced for the development of HCV vaccines. An extensive number of studies have been published on these vaccines, and some vaccines were even tested in clinical trials. In the current review, progress in the development of a preventive and therapeutic vaccine against the hepatitis C virus is reviewed in the context of Peptide vaccines, Recombinant protein vaccines, HCV-like particle, DNA vaccines and Viral vectors expressing HCV genes.

**Key words:** Preventive vaccine; Therapeutic vaccine; Hepatitis C virus; Hepatitis C virus infection

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**Core tip:** Chronic hepatitis C virus (HCV) infection occurs in about 75%-90% of acutely infected individuals. It may progress to liver cirrhosis or hepatocellular carcinoma. Despite satisfactory progress in the management and treatment of chronic hepatitis C, it remains one of the most prominent viral infections worldwide. No reliable vaccine for it has yet been developed; however, researchers are trying to design and develop different types of vaccines to prevent HCV infection or to cure the chronic form of the disease. The current article provides an overview of the latest progress in the development of preventive and therapeutic vaccines against HCV infection is reviewed in the context of Peptide vaccines, Recombinant protein vaccines, HCV-like particle, DNA vaccines and Viral vectors expressing HCV genes.

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# Introduction

The hepatitis C virus (HCV) is an enveloped, positive-sensed, single-stranded RNA virus belonging to the *hepacivirus* genus of the *Flaviviridae* family[1]. Approximately 75% of acute HCV cases develop into chronic HCV infection, of which 3%-11% develop into liver cirrhosis within 20 years. This may eventually lead to liver failure or hepatocellular carcinoma (HCC) and may necessitate the need for a liver transplant[2-4].

HCV is mainly transmitted through blood as in transfusion, injection drug use, organ transplantation, hemodialysis, or accidental exposure; however, unprotected sexual contact and vertical mother-to-child transmissions have also been documented[5,[6](#_ENREF_6)].

First discovered in 1989[7], it is estimated that 3% of the world's population is infected with HCV. Infection rates vary from less than 1% to over 10% in different countries[8]. HCV antibody (anti-HCV) testing is used to assess the prevalence of HCV. Studies have reported that countries located in Africa and Asia have the highest anti-HCV rates, whereas industrialized countries such as those located in North America, Western Europe, and Australia have lower rates[9-11]. Although the incidence rate of HCV is decreasing in developed countries, mortality due to secondary liver disease from HCV infection is expected to continue to rise over the next 20 years[12]. HCV infection is more common in adult populations (people older than 15 years)[13]. With efficient screening strategies, better treatments, and the use of preventive vaccines, it is estimated that HCV could be eliminated in the next 15 to 20 years[13,[14](#_ENREF_14)].

HCV strains are categorized into seven genotypes (1-7) based on their phylogenetic similarities and genome sequence. HCV genotype 1 is the most common, accounting for 46.2% of all cases worldwide, and followed by genotype 3 as the second most prevalent genotype which is responsible for 30.1% of all cases[15].

The majority of newly-infected people are asymptomatic or have a mild illness. Therefore, they are normally not aware of their condition[4]. Symptoms are anorexia, nausea, vomiting, abdominal discomfort, and jaundice. HCV rarely causes fulminant hepatic failure[16].

Drug therapy for HCV depends on the administration of interferon alpha (INF-α) and ribavirin and is associated with adverse side effects. A new class of drugs called direct-acting antivirals (DAA) is beginning to be used in combination with INF-α and ribavirin, resulting in an increase in their effectiveness; however, the setbacks are high costs and more adverse side effects such as fever, fatigue, chills and depression[15]. Taking into account these factors and the fact that only a small percentage of patients can be totally cured[17], the need for an effective HCV vaccine is apparent. The present review aimed to review the obstacles and progress in the challenge of developing effective vaccines against the hepatitis C virus.

# HCV Virology

HCV has an icosahedral capsid that consists of a single-stranded, positive-sensed RNA and is enveloped with E1 and E2 glycoproteins which are highly variable[18]. Scientists have been unable to culture HCV in vitro. This, in turn, makes studying its structure and replication processes difficult. Nevertheless, some progress has been made in this regard[19,[20](#_ENREF_20)]. Virus replication occurs mainly in hepatocytes. It is estimated that each infected cell produces fifty virions daily. The virus also replicates in peripheral blood mononuclear cells and may be responsible for high levels of immunologic disorders in chronic hepatitis C patients[1].

The genome contains a long open reading frame (ORF) encoding a 3000 amino acid length polyprotein that is cleaved into structural and non-structural proteins. A 5′ untranslated region functions as an internal ribosome entry site and permits direct binding of ribosomes to the start codon of the ORF, thus directing translation of HCV's ORF[21,[22](#_ENREF_22)]. The first about 40 nucleotides of the RNA genome may be involved in RNA replication[23]. The 3′ untranslated region is comprised of four elements: (1) a short variable sequence; (2) a poly(U) tract of heterogeneous length; (3) a polypyrimidine stretch mainly containing U and a few Cs; and (4) a highly conserved 98-nucleotide sequence[24,[25](#_ENREF_25)]. The poly(U) tract and the highly conserved region of 3' UTR are essential for the infectivity of HCV[26].

Viral structural proteins include core protein (22 kDa), E1 (33 kDa), and E2 (70 kDa) glycoproteins, and non-structural (NS) proteins are namely NS2, NS3, NS4A, NS5A, and NS5B[27,[28](#_ENREF_28)]. HCV core is a 22 kD basic protein responsible for forming the capsid structure. It has been implicated in HCV pathogenesis, the development of chronic infection, and immune response modulation. Core is the most conserved protein among various HCV genotypes, and humoral responses against it are the first host responses to develop in an infected patient[29-31]. E1 and E2 play roles in cell entry. E2 glycoprotein interacts with the HCV receptor CD81 in liver cells. Moreover, low density lipoprotein (LDL) receptors, scavenger-receptor class B type 1 (SR-B1), and Claudin-1 act in the process of cell entry[18]. E2 contains three hypervariable regions (HVR) that play roles in cell entry, antibody binding, and outcome of the disease. In HCV genome, P7 gene encodes the first non-structural (NS) protein and is located after the genes which encode structural proteins. It forms the ion channels that seem to be essential for HCV efficient assembly, release and production[32].

P7 is the first non-structural (NS) protein located after structural proteins. Its function is unknown. It forms the ion channels that seem to be essential for HCV production[19,[29](#_ENREF_29)]. NS2 (23kDa), which is located early after P7, is a membrane-associated protease which can cleave the NS2-3 junction. P7 has been shown to cooperate with other non-structural proteins and may facilitate viral assembly[29,[33](#_ENREF_33)]. NS3 (67k Da) is a serine protease with RNA helicase and NTPase activities that cleaves HCV polyprotein at various junctions between non-structural proteins[29,[33](#_ENREF_33)]. NS3 also regulates NS2 protease activity and inhibits type 1 interferon (IFN) production by blocking Toll-like receptor 3 (TLR3) and retinoic acid-inducible gene 1 (RIG-I) signaling pathways[34,[35](#_ENREF_35)]. NS4 is further cleaved into NS4A and NS4B. NS4A plays a role in the assembly of the HCV replication complex, viral pathogenesis, modulation of NS3 helicase, and facilitation of NS3 serine protease activities[29,[33](#_ENREF_33)]. NS4B is responsible for the formation of cellular membranous webs which are the site of genomic replication in HCV. Any allelic variation in its sequence may strongly affect HCV replication[36].

The NS5A protein has RNA-binding activity and directly binds to NS5B, modulating its RNA polymerase activity[29,[33](#_ENREF_33),[37](#_ENREF_37)]. The NS5B protein is an RNA-dependent RNA polymerase that lacks proofreading activity, thus giving rise to a high rate of replication errors, and generates significant genetic diversity. This high rate of genetic variability produces a mixture of variants called quasispecies, even in a single infected individual[38].

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# Natural immune responses to HCV

Figure 1 summarizes immune responses against HCV with a focus on both innate and adaptive immune responses. More details about the components and effects of such responses are found in the following sections (3.1-3.3).

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## *Innate immunity and HCV*

Innate immune, the first system to respond to HCV infection, contains proinflammatory cytokines and a cellular component. In addition to resisting infection, innate immunity is also involved in provoking adaptive immunity[39]. Three arms of the innate immunity that identifies HCV infection as a threat are: (1) retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs); (2) toll-like receptors (TLRs); and (3) nucleotide oligomerization domain (NOD)-like receptors (NLRs)[40]. After HCV recognition, these arms send various downstream signals to induce the production of various cytokines, such as interleukins (IL) and IFNs, which create an antiviral state for uninfected cells, decrease HCV replication in infected cells, and link innate immunity to adaptive immunity[39]. The RIG-I pathway is activated shortly after infection. HCV promotes a structural change in RIG-I and leads to the activation of transcription factors IRF3 and NFκB, which results in the production of type I and III IFNs[41].

TLRs can sense both viral nucleic acid and protein. TLR3 and 7 have been shown to play roles in intracellular HCV recognition[42]. TLR3 is found in hepatocytes and Kupffer cells and inhibits HCV replication[43,[44](#_ENREF_44)]. TLR7 resides in plasmacytoid dendritic cells (pDCs) and Kupffer cells, leading to the production of type I and III IFNs and IL-1β and IL-18[45,[46](#_ENREF_46)].

The NLR pathway results in the activation of inflammasome, a protein complex made of a sensor (NLR protein 3), the adaptor (ASC), and caspase 1 (a cellular protease)[47]. NLRs trigger the secretion of IL-1β and IL-18 which cause hepatic stellate cells to form fibrosis[48].

IFNs-α and β (type I IFNs) bind to similar receptors and activate the Jak/Stat pathway, which in turn induces interferon-stimulated genes (ISGs). This amplifies total IFN responses and degrades viral RNA[49]. Type III IFNs include IFN-λ 1, 2, and 3 (IL-29 and IL-28 A and B, respectively). They activate the Jak/Stat pathway and induce ISGs, the same as type I IFNs; however, they bind to different receptors with distinct cellular distribution[50].

The role of IL-18 is still unclear in HCV infection, but it may suppress virus replication and induce the maturation of monocyte-derived dendritic cells (DCs)[51]. Plasmacytoid DCs (pDCs) may be the major source of IFN-α production. Myeloid DCs (mDCs) migrate to lymphoid tissue to activate native T cells and bridge innate and adaptive immune responses[45].

Natural killer (NK) and NKT cells also play roles in HCV infection through IFN type II (IFN-γ) production and cytotoxic destruction of infected cells[52,[53](#_ENREF_53)]. IFN-γ activates Kupffer cells, and they release proinflammatory molecules such as tumor necrosis factor (TNF)-α, galectin-9, and IL-18[51,[54](#_ENREF_54)].

## *Humoral immunity and HCV*

Humoral responses to HCV infection include B cell activation and production of antibodies which are primarily low titers of IgG1 and appear late in the course of the disease[55,[56](#_ENREF_56)]. Antibodies can be detected after 7-10 wk of infection; however, viral RNA can be detected 1-3 weeks after infection[57].

Although a few antibodies for E1 glycoprotein as well as non-structural proteins have been found, the main target for antibodies are the surface E2 glycoprotein[56,[58](#_ENREF_58),[59](#_ENREF_59)]. Antibodies against NS proteins were found earlier in the disease course and with a greater magnitude. Nevertheless, E2 glycoprotein is still considered the main target for antibodies. Recent studies have suggested that patients infected with HCV show an elevated level of activated B cells, and people with defects in antibodies experience rapid disease progression, emphasizing the role of humoral immunity in HCV[60-62]. Patients with primary hypogammaglobulinemia could spontaneously resolve acute hepatitis C, suggesting that humoral immunity is essential for HCV clearance[63].

Recent studies have suggested that patients infected with HCV show an elevated level of activated B cells, and people with defects in antibodies experience rapid disease progression, emphasizing the role of humoral immunity in HCV[60-62]. Patients with hypogammaglobulinemia could spontaneously resolve acute hepatitis C, suggesting that humoral immunity is not essential for HCV clearance[63]. The activity of humoral immunity may contribute to some of the extra hepatic disorders associated with HCV such as mixed cryoglobulimia and B-cell non-Hodgkin's lymphoma, although the mechanisms of these associations are still not well understood[64,[65](#_ENREF_65)].

## *Cell mediated immunity and HCV*

Cell immunity for HCV is composed of two arms: CD4+ and CD8+ T cells that are detectable in peripheral blood or the liver several weeks post-infection[66]. These T cells directly kill infected cells or produce soluble factors that clear the virus in a non-cytolytic manner. These cells may also directly damage the liver or attract non-specific inflammatory cells to accelerate liver inflammation[67].

Upon recognition of HCV through the major histocompatibility complex (MHC) class II[68], CD4+ T cells (T helper) mainly produce IFN-γ that correlates with decreased HCV titer and transaminase levels[66]. These cells also produce IL-2 that helps the production of CD8+ (cytotoxic) T cells. Moreover, they can enhance B cell production. T helper actions are essential for spontaneous recovery during acute HCV[69]. It has been shown that T helper responses are not observed in patients developing chronic disease[70].

CD8+ T cells recognize HCV-infected cells through MHC class I and lead to lysis of the infected cells[68]. The role of MHC class I in HCV recognition indicates that spontaneous clearance of HCV is related to the presence of some MHC class I molecules, including HLA-B\*57, HLA-B\*27, HLA-A\*11, HLA-A\*03, and HLA-Cw\*01, and to the absence of HLA-Cw\*04[71]. The mechanism of the influence of these alleles in the clearance of HCV is unknown; however, some studies suggest binding proteins which are produced by these allelic variants of protective genes to immunogenic or functionally conserved T cell epitopes[72]. There is evidence for the binding of some proteins to inhibitory receptors of NK cells. MHC class II genes, such as DQB1\*0301 and HLA-DRB1\*1101, have also been associated with HCV clearance. Patients who lack efficient cytotoxic T cell response develop persistent hepatitis C infection, but the magnitude of the cytotoxic T cell response is associated with the clinical outcome[73]. Patients who lack efficient cytotoxic T cell response develop persistent hepatitis C infection, but the magnitude of the cytotoxic T cell response is not associated with the clinical outcome[74].

# Challenges to developing a HCV vaccine

## *HCV genetic variability*

Studies suggest that the hepatitis C virus first appeared over 1000 years ago and evolved in different environments into 7 distinct genotypes and more than 100 subtypes[15,[75](#_ENREF_75),[76](#_ENREF_76)]. HCV mutates at a rate of nearly 1 nucleotide per replication cycle. This is due in part to the lack of proofreading activity of NS5B RNA-dependent polymerase that causes an error rate of 10−3 to 10−5 per nucleotide per replication cycle[19]. These frequent mutations, in combination with a short viral half-life and rapid turnover (1010 to 1012 virions per day), lead to a high genetic variability that results in the presence of distinct but closely related HCV variants (known as quasispecies) in 1 infected individual[38]. E1 and E2 glycoproteins show the highest variability among HCV proteins[75]. It is estimated that HCV is 10 times more variable than the Human Immunodeficiency Virus (HIV), and this clearly poses a significant challenge for successful vaccine development[77].

## *Immune evasion and HCV persistence*

HCV can escape the immune system by inhibiting NK cells and IFN-α production and by mutating to evade antibody and CD8+ T cell recognition. The mutations in CD8+ T cell epitopes have been carefully described in many chimpanzee[78,[79](#_ENREF_79)] and human studies[80-82].

During HCV replication, NS3 and NS4A block the signaling pathways of TLR3 and RIG-I, and that ultimately inhibits the production of type I IFN[83-85]. This may lead to a decrease in NK cell activity through a lack of IL-15 production[86,[87](#_ENREF_87)]. Core protein interferes with TNF-α and lymphotoxin signaling[88], and E1 and E2 glycoproteins build a glycan shield and facilitate cell-to-cell spread[89]. The NS5A protein could stimulate IL-8, inhibit double-stranded RNA-activated protein kinase (PKR), and interfere with 2',5'-oligoadenylate synthetase (2',5'-OAS), thereby interrupting the signaling of type 1 interferon[90,[91](#_ENREF_91)]. The overexpression of HCV core protein interacts with the STAT1 SH2 domain and leads to the inhibition of IFN signaling[92,[93](#_ENREF_93)]. The upregulation of ubiquitin-specific peptidase 18 (USP18) due to long-term IFN stimulation suppresses ISG15 activation and Jak/STAT signaling, which can lead to type I IFN stimulation resistance[94,[95](#_ENREF_95)]. Natural killer and natural killer T-lymphocyte cells are present in liver tissue and produce IFN-γ and other cytokines to prime cellular immune responses[96].

HCV escapes humoral immunity by several mechanisms: (1) HCV binds to very low density lipoprotein (VLDL), which facilitates its uptake by hepatocytes[97]; (2) three glycans at the CD81 binding site of E2 glycoprotein decrease the immunogenicity of the virus[98]; (3) CD81 and Claudin-1 allow HCV to infect surrounding cells through cell-to-cell contact[99]; and (4) constant mutations in HCV can induce interfering antibodies[100].

Another obstacle in developing an efficient vaccine is the persistence of HCV, mainly because of (1) rapid HCV escape mutations[101]; (2) the secretion of immune regulatory cytokines[102]; (3) T cell exhaustion and deletion[103,[104](#_ENREF_104)]; and (4) T regulatory cell induction[105]. A further obstacle is the lack of a suitable animal model to study HCV.

HCV polymerase works at a high rate and lacks proof-reading activity, which leads to a rapid viral escape from humoral and cellular immunity[106,[107](#_ENREF_107)]. Mutations in MHC class I restricted epitopes which are targeted by cytotoxic T cells may lead to persistence[108,[109](#_ENREF_109)].

IL-10, an immune-regulatory cytokine, increases in chronic hepatitis C. It is produced by T cells as well as monocyte and natural killer cells. IL-10 not only suppresses IFN-α and IFN-γ production and T cell proliferation, but also promotes plasmacytoid dendritic cell apoptosis[110], resulting in liver damage in patients with chronic hepatitis C virus infection[104]. Tumor growth factor (TGF)-β also plays a role in HCV persistence[102].

In T cell exhaustion, T cells are in a state of dysfunction. This phenomenon can develop during many chronic infections like HCV. These cells lose their ability to produce IL-2 which is essential for the production of T cells and show sustained expression of inhibitory receptors. This results in a loss of cytotoxicity and the cytokine production of cellular immunity[111]. Programmed cell death protein-1 (PD-1) plays important roles in CD8+ T cell exhaustion, and blocking PD-1 restores CD8+ T cell responses[112-114]. T lymphocyte Antigen 4 (CTLA4) also contributes to T cell exhaustion. Inhibition of both PD-1 and CTLA4 improves the restoration of T cell responses[115]. HCV induces T cell deletion by upregulating the pro-apoptotic molecule Bcl-2-interacting mediator (Bim) and downregulating the induced myeloid leukemia cell differentiation protein (Mcl-1)[104].

Regulatory T cells control the balance between host damage and viral control. These cells can induce immune-tolerance when excessive immune response could be harmful for the host. They inhibit the maturation of antigen presenting cells and the activation of T cells. Thus, they control auto-immunity and immune responses[116]. Although higher T regulatory cell frequency has been observed in chronic HCV compared to self-resolving infection[117-119], a study in chimpanzees showed that HCV infection increases the number of T regulatory cells and their suppressive actions irrespective of the disease outcome[120].

HCV attacks mitochondria in liver cells and induces mitochondrial dysfunction and oxidative stress[121-123]. This results in the induction of cell autophagy and mitophagy, selective elimination of mitochondria, and the induction of mechanisms to eliminate dysfunctional mitochondria[124]. Autophagy proteins act as proviral factors that initiate the translation of HCV RNA in newly-infected cells, and autophagosomes will become sanctuaries for HCV replication away from host immune surveillance, which paves the way for chronic infection and liver failure[125,[126](#_ENREF_126)]. Kim *et al*[127] suggested a similar role for mitophagy in the HCV life cycle.

## *Lack of an appropriate HCV model*

Other than humans, chimpanzees[128] and a non-rodent small mammal named *Tupaiabelangeri*[129], also known as the tree shrew, are naturally susceptible to HCV infection. In addition to the mentioned animal models, much effort has been made to introduce genetically-manipulated animals, preferably mice or rats, for the study the HCV pathogenesis and vaccine design[130-133]. The results of chimpanzee studies are highly variable and difficult to interpret because of the genetic variability between animals and small sample sizes. 30%-40% of chimpanzees develop chronic HCV infection, whereas up to 85% of infected humans develop chronic HCV[134]. The high cost of acquiring and maintaining the animals, their limited availability, and ethical considerations are major drawbacks to using chimpanzees as HCV animal models[133]. The infection rate and viremia of tree shrews is low and rarely sustained; however, they can develop chronic hepatitis in some cases[135,[136](#_ENREF_136)]. The limited availability of tree shrews, their high housing costs, and the lack of tupaia speciﬁc reagents (used to assess HCV-host interactions) limit their use for the study of HCV[128].

HCV is a highly complex virus. The study of immune responses was long hampered by the lack of a cell culture system or readily accessible small animal model. HCV can replicate in lymphoid or peripheral blood mononuclear cells (PBMC), but only for limited periods of time and very low viral loads[137]. The invention of HCV pseudo-particles (HCVpp) expressing unmodiﬁed E1E2 glycoproteins has provided a way for more thorough studies of HCV-speciﬁc antibodies[138], although the structure and neutralization of HCVpp are signiﬁcantly different from natural HCV[139].

The first successful tissue model of HCV infection was developed in 2005 using the HCV/JFH1 cell culture system[140]. Whole genome of The JFH1 virus was separated from a Japanese patient with fulminant hepatitis, it was then multiplied with PCR, cloned and was named JFH1. The JFH1 virus is one of the 2a genotype, has a full-length HCV genome. This culturing system efficiently maintains the replication of hepatitis C virus in human hepatoma cell line Huh7 and produces fair titers of cell-cultured derived HCV particles (HCVcc) with natural infectious properties. This platform was further broadened to include HCV subtype 1a strain H77-S that is more associated with HCV complications such as hepatocellular carcinoma (HCC) and cirrhosis and also more resistant to IFN therapy[141].

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# Preventive and therapeutic vaccines

Any preventive (prophylactic) or curative (therapeutic) immunogen that inhibits or treats the chronic phase of HCV infection can be referred to as an effective HCV vaccine, since HCV persistence results in liver failure and the development of clinical complications[142]. Patients reinfected with HCV demonstrated a significantly increased spontaneous viral clearance rate and broader T-cell responses compared with those who had been affected with primary infections[143]. Differences in viral kinetics and rates of clearance between primary and secondary infections strongly support the significance of memory immune responses in the clearance of natural infection and support the development of therapeutic and prophylactic vaccines[143,[144](#_ENREF_144)].

Most chronic HCV infections exhibit lower HCV-specific T-cell responses compared with humoral responses. Thus, efforts have focused more on enhancing the T cell rather than humoral responses. In addition to inducing strong and cross-reactive neutralizing antiviral antibodies (humoral immunity), a suitable HCV vaccine should provoke long-lasting cellular immune responses involving helper CD4+and CD8+ T cells (cellular immunity)[31,[145](#_ENREF_145)]. Although current therapeutic vaccine trials have successfully induced HCV-specific immune responses and transiently reduced viral RNA in subsets of patients, they have not completely cleared HCV infections or constantly reduced viral titers[145-147]. In the current study, different vaccine development strategies and the clinical trials in which they were tested were reviewed (Table 1).

## *Peptide vaccines*

Peptide vaccines induce HCV speciﬁc T cell responses by presenting vaccine peptide to the T-cell receptor *via* HLA molecules. Therefore, they are HLA-specific and target only a selected subset of epitope sequences within the HCV genome. The high rate of genetic variation in viruses within populations and geographic regions limits the universal usage of these vaccines. They often contain multiple epitopes in order to induce broader T cell responses. Unfortunately, some peptides may induce T regulatory cells or immune tolerance[148].

Intercell (Intercell AG, Vienna, Austria) developed a peptide vaccine, IC41, which consists of 5 synthetic peptides derived from conserved regions of core, NS3, and NS4 proteins of HCV genotypes 1 and 2 with a poly-L-arginine adjuvant. IC41 was assessed in 128 HLA-A2+ healthy volunteers in a phase I, dose-escalation, placebo-controlled randomized trial where it was proven to be safe and well tolerated. IC41 vaccination induced few interferon-producing cells as well as dose-dependent T cell immune responses[149]. Klade *et al*[146] sought to determine whether IC41 is able to induce HCV-specific T-cell responses in chronic hepatitis C patients. In their study, 60 HLA-A2+ chronic HCV patients failing to respond to or relapsing from conventional therapy were sorted randomly into 5 groups to receive 6 doses of IC41 in a double-blind phase II study. T-cell proliferation was recorded in up to 67% of patients, and IFN-gamma responses were observed in up to 42% of patients in the IC41 vaccine groups. Moreover, 3 patients had transient declines of HCV serum RNA. Another randomized trial included 54 healthy subjects receiving either subcutaneous or intradermal IC41 vaccinations weekly or bi-weekly. Results showed that IC41 induced significant immunological responses; adding imiquimod to the formulation did not enhance immunogenicity and was associated with a lower immune response. Furthermore, intradermal injections caused more noticeable reactions, especially erythema and edema[150]. In another clinical trial conducted by Klade *et al*[151], 50 patients received 8 intradermal IC41-plus-imiquimod vaccinations bi-weekly and 21 patients received 16 subcutaneous vaccinations without imiquimod weekly. The first group showed a statistically significant HCV viral load decline, whereas no effect on HCV viral load was observed in the second group. A team in Japan also worked on HCV peptide vaccines and published two papers[145]. The first study assessed the functionality of a "personalized" vaccine containing four CD8+ A24 peptides combined with Freund’s adjuvant in 12 HCV patients (genotype 1b) who had previously failed in standard interferon-based therapy. After the first vaccination, the rest of the vaccinations were carried out with only those peptides that produced responses in each participant. Although most patients developed peptide-specific T cell responses after the seventh injection, the authors observed a dose-dependent decrease in serum ALT and HCV RNA levels in only 5 and 3 patients, respectively[145]. In the second study, another peptide vaccine composed of HCV core region (C35–44) peptides with ISA51, an emulsiﬁed incomplete Freund’s adjuvant, were shown to be safe and well tolerated in a phase I trial on 25 HCV non-responder patients. Around 60% of patients showed an increase in peptide-specific T cytotoxic responses. Also, a more than 30% improvement in ALT and a > 1 log reduction in viral load were observed in 7 and 2 patients, respectively[152].

A phase I, dose-escalation, placebo-controlled randomized control trial was performed to assess a virosome-based peptide vaccine containing NS3 peptides in 30 healthy participants; however, no results have been released so far (ClinicalTrials.gov Identiﬁer: NCT00445419).

## *Recombinant protein vaccines*

Recombinant protein vaccines are developed by isolating the gene(s) encoding the corresponding protein and cloning it in bacteria, yeast, or mammalian cells. This approach is based on the theory that an efficient number of viral epitopes can induce enough immune responses to develop protective immunity, generally including antibodies and CD4+ T cell responses. Some recombinant proteins are, by themselves, sufficient for developing a strong immune response, whereas others require adjuvants. The advantages of recombinant protein vaccines are that they do not contain the pathogen or its genetic material and no organism culture is required.

In 1994, a recombinant heterodimeric E1E2 vaccine was tested on 7 chimpanzees and showed sterilizing immunity against a homologous HCV strain in 5 of them (Table 1)[153]. This vaccine was further tested against persistent infection by homologous and heterologous HCV strains and offered protection in 10/12 and 8/9 chimpanzees, respectively[154]. Puig *et al*[155] vaccinated 2 chimpanzees, 1 naive and 1 recovered from acute HCV infection, with recombinant HCV E1E2 glycoproteins. High antibody titers to E1E2 in addition to strong T cell proliferative responses were observed. After challenge with HCV, viremia was delayed in both vaccinated animals compared to the non-immunized animals. The naïve chimpanzee became persistently infected, despite initial strong immune responses, and the other chimpanzee had a significantly shorter and milder viremia compared to the re-infection of the non-vaccinated animals. Many other studies evaluating the efficacy of recombinant HCV vaccines in animals, especially chimpanzees, achieved similar results which paved the way for recombinant HCV vaccine trials in humans (Table 1)[156-159].

The first prophylactic HCV vaccine tested in humans was a C-terminally shortened recombinant E1 protein with aluminum hydroxide (alum) adjuvant named T2S-918/InnoVac-C. This vaccine provoked a higher antibody response against E1 in healthy volunteers than in patients with persistent HCV infection. Studies on this vaccine, however, were ceased in 2007 (Table 1)[160].

Another recombinant E1E2 heterodimer vaccine with MF59C adjuvant was given to 60 healthy subjects in 3 different doses on day 0 and weeks 4, 24, and 48. Neutralizing antibodies and T cell responses to E1/E2 were observed in all subjects. Moreover, the produced antibodies were shown to block CD81, a major entry molecule for HCV. Although the vaccine was safe and well tolerated, its usage is hampered due to technical difficulties in E1E2 protein manufacturing (Table 1)[161].

In 2011, Verstrepen *et al*[162] vaccinated 4 chimpanzees with either genotype 1b E1 or E2 recombinant with alum adjuvant and observed antibody responses in all subjects. Only antibodies against E1, however, were shown to neutralize HCV pseudo-particles. Furthermore, only chimpanzees vaccinated with E1 were protected from persistent hepatitis when challenged with a 1b strain of HCV.

Drane *et al*[163] evaluated a recombinant HCV core protein vaccine with ISCOMATRIX™ adjuvant. After satisfying results were obtained in rhesus macaques, a phase I, placebo-controlled, dose-escalation clinical trial was conducted on 30 healthy volunteers. All participants except 1 showed antibodies against HCV core protein. Despite the induction of strong CD4+ and CD8+ T cell responses in monkeys, T cell responses were observed in only 2 subjects who received the highest dose of vaccine. T cell cytokines were detected in all but 1 of the participants in the highest dose group.

InnoVAC-C, developed by Innogenetics Company (Innogenetics NV, Ghent, Belgium), was the first HCV therapeutic vaccine candidate based on recombinant E1 protein in alum adjuvant. The vaccine was administered to 26 of 35 chronically infected HCV patients (genotype 1) at weeks 0, 4, 8, 12, and 24, and others received a placebo (alum only). Then, 34 subjects received open-label E1 vaccines at weeks 50, 53, 56, 59, 62, and 65. Twenty-four subjects underwent liver biopsies before E1 vaccination and 17 mo later. According to Ishak scores, 9/24 patients (38%) improved 2 points or more, whereas others remained stable or their condition was exacerbated. The increase in anti-E1 specific antibody levels correlated with the relative decrease in alanine aminotransferase (ALT) level. A significant de novo E1 specific T cell response was observed in all but 3 subjects[164]. Finally, studies on this vaccine led to a larger, placebo-controlled trial for which 122 chronically HCV infected patients were randomly chosen to receive 4 courses of 6 injections over 3 years. Vaccination induced anti-E1 humoral and cellular immune responses, but contrary to earlier findings, the histological progression of liver disease was not halted[165]. The Innogenetics company in Belgium eventually abandoned this HCV vaccine program in 2008, and no further work has been published[166].

Another therapeutic vaccine, GI-5005, is based on recombinant core and NS3 proteins of HCV produced in yeast cells (*Saccharomyces cervisiae*). Studies of *in vitro* and *in vivo* models demonstrated the robust immunogenicity of GI5005. Moreover, GI-5005 was evaluated in a phase I b clinical trial and displayed efficacy in patients with chronic HCV infection[167]. GI5005 was evaluated in combination with the standard therapy (PEG-IFN/ribavirin) in more than 250 chronic HCV-1 patients in a phase II, placebo-controlled trial. Triple therapy was well tolerated with no increase in withdrawals due to adverse effects. Moreover, improved early virological responses were observed in all treated naïve patients as well as an increase in sustained virological response rates in prior non-responder patients[168].

## *DNA vaccines*

In 2001, the first DNA vaccine was licensed for use to protect horses from West Nile virus[169]. The injection of a plasmid encoding antigenic HCV protein(s) or peptide epitope(s) could result in protein expression *in vivo,* leading to both humoral and cellular immune responses in rhesus macaques[170-172]. DNA vaccination against HCV primes periphery T cells, which subsequently enter the liver and help clear the infection. Unfortunately, the initial success with the DNA vaccination in mice did not continue in humans, possibly because as the size of the immunized host increases, the efficacy of DNA uptake and gene expression decreases[173,[174](#_ENREF_174)]. DNA vaccine includes the nucleotides encoding an antigenic portion of the virus such as the viral core region or envelope region. The DNA vaccine is taken up in to the host cell, translated and the protein is produced. These proteins are processed via the endogenous MHC class 1 pathway and promoted cell-mediated immunity (CMI).The first DNA-based vaccine to reach clinical trial for HCV infection was CIGB-230, which contained a mixture of Core/E1/E2-expressing plasmid with HCV core protein. The phase I trial of this vaccine resulted in neutralizing antibody and HCV core speciﬁc T cell responses in the majority of patients. Furthermore, nearly half of the vaccinated individuals developed an improvement in liver histology with a reduction in ﬁbrosis, even though viremia persisted[175]. Another clinical trial for CIGB-230 on 15 chronic HCV patients not responding to IFN treatment indicated that 6 patients developed weak de novo neutralizing antibody responses, and only 1 patient had a drop of > 1 log10 in viral load[176].

Due to the heterogeneity of HCV subtypes, a DNA vaccine including the most conserved regions (NS3 and NS4a) was designed. Through extensive codon modiﬁcation, the DNA can be effectively expressed *in vivo* and prime Th1 and CD8+ CTL responses in transgenic mice models[177,[178](#_ENREF_178)]. This vaccine, named ChronVac-C and generated by the Tripep Company of Sweden, was the second DNA-based HCV vaccine to reach a human trial, in which it was given to 12 chronic HCV patients through intramuscular electroporation. Initial results suggested safety and immunogenicity of vaccine. Two out of 3 patients receiving the highest dose showed a decrease in serum HCV RNA. Also, 3 patients who were under standard IFN-based therapy had an accelerated viral load clearance. Therefore, a treatment combining ChronVac-c with the standard IFN-based therapy was proposed for chronic HCV patients[179]. This vaccine is now in phase II clinical trials for HCV infection (ClinicalTrials.gov Identiﬁer: NCT01335711).

The nonstructural protein 3 (NS3) of the hepatitis C virus, an attractive candidate for use in HCV vaccination, plays an important role in the viral life cycle while simultaneously interfering with the host defense system. NS3's serine protease cleaves the mitochondrial antiviral signaling proteins, thereby blocking IFN-β production[180-183]. NS3's nucleoside triphosphatase (NTPase)/RNA helicase activities may interfere with cellular RNA helicase-mediated functions, such as DNA replication, RNA transcription, and other cellular phenomena[183,[184](#_ENREF_184)]. To overcome this challenge, Ratnoglik *et al*[185] constructed a series of DNA vaccines that express NS3 of HCV but with mutations to the catalytic triad of the serine protease and the NTPase/RNA helicase domain to eliminate the problems mentioned above. Immunization of BALB/c mice with resultant DNA vaccine candidates induced T cell immune responses similar to those induced by the wild type NS3 or NS3/4A complex.

## *Viral vectors expressing HCV genes*

The main idea for vector-based vaccines is the use of manipulated viruses to deliver foreign genetic material to mammalian cells. Viruses can enter cells and express desired genes within the host while becoming non-pathogenic and non-replicative by deleting specific genes. Compared to Peptide vaccines, vector-based vaccines introduce a broader range of viral epitopes and are presented through the MHC-I pathway and induce broader CD4+ and CD8+ T cell responses[186]. Adenoviral (Ad) vectors are the most used viral vectors for T-cell priming in non-human primates (NHPs) and humans[187]. Studies of HIV vaccination on rodents and non-human primates established the use of adenoviral vectors as the most efficient approach to prime cytotoxic T cell responses[188]. Adenoviral vaccines can stably express large foreign genes, remain outside the host's chromosome, and become replication defective by deleting the E1 locus[186,[189](#_ENREF_189)]. However, one major drawback to using adenoviral vectors is a pre-existing immunity to them that results in vector clearance before inducing the desired immune response. To overcome this problem, rare subtypes, non-human adenoviruses (which are harmless to humans), or adenoviruses with altered surface proteins are used[187].

An Ad6-based vaccine, encoding the NS3, NS4A, NS4B, NS5A and inactivated NS5B, was capable of inducing strong and specific T-cell responses against non-structural (NS) antigens of HCV in mice and rhesus macaques[190]. Okairos, Inc. (Rome, Italy) further investigated this vaccine by testing it on 5 chimpanzees. Four chimpanzees showed protective levels after being challenged with a heterologous HCV virus due to the induction of cross-reactive cellular responses[191]. After the safety of the Okairos vaccine was confirmed in a phase I study[192], another trial was directed, but no results have been published yet (ClinicalTrials.gov Identifier: NCT01070407).

On the strength of this preclinical data, Barnes *et al*[193] conducted a phase I clinical trial to assess chimpanzee Ad3 (ChAd3) and Ad6 vectors in 36 healthy volunteers where both vaccines induced specific T cell responses against multiple HCV proteins. These responses were capable of recognizing heterologous HCV strains (genotypes 1A and 3A). However, the vaccine was not as effective as predicted from the results in rhesus macaques, possibly due to higher levels of cross-reactive antibodies against Ads in humans[193,[194](#_ENREF_194)].

Another approach in developing vector-based vaccines is the use of modiﬁed vaccinia Ankara (MVA) viruses to encode HCV speciﬁc genes. They are a highly attenuated poxvirus strain used in several vaccine designs for conditions including colorectal cancer, TB, HIV, and melanoma[195-198]. The MVA genome is incapable of integrating into host genome as its life cycle takes place entirely in the host cytoplasm. Its use is more efficient than Ad-based vectors due to minimal pre-existing anti-MVA immunity[199,[200](#_ENREF_200)].

The Transgene Company vaccinated chimpanzees with a heterologous prime-boost regimen with DNA and MVA encoding core, E1, E2, and NS3 genes (Table 1)[201]. Immunization induced strong HCV speciﬁc CD4+ and CD8+ T cell responses. In addition, all animals receiving prime-boost vaccines achieved high HCV-speciﬁc antibody titers, whereas those receiving the DNA prime alone did not. When challenged with a heterologous strain of HCV, 3 out of 4 chimpanzees developed chronic infection[201].

An MVA-based vaccine encoding HCV NS3, NS4, and NS5B genes was able to induce strong long-lasting cross-reactive HCV speciﬁc helper and cytotoxic T cells in HLA-A2.1 and HLA-B7.2 transgenic mice. An additional dose of vaccine given after 6 mo boosted these responses, suggesting that the vaccine can induce effective memory T cells[202]. This vaccine, also called TG4040, was evaluated in a dose-escalation trial of 15 chronically infected HCV patients. Results showed that 6 patients had a decline in HCV viral load (0.5–1.4 log10) which was associated with a significant CD8+ T-cell response[203]. A phase II trial using the TG4040 vaccine in combination with standard PEGylated interferon and ribavirin therapy has been completed, but no results have been published yet (ClinicalTrials.gov Identifier: NCT01055821).

Vector-based vaccines with strategies using neither modiﬁed vaccinia Ankara viruses nor adenoviruses are also under development. A study in HLA-A2.1-transgenic mice used the canary pox virus as a viral vector of 2 plasmids coding entire HCV genome to boost the primary immunization with HCV DNA vaccine. Two months after a canary pox virus boost, strong cellular immune responses to HCV proteins were observed[204]. Another study also evaluated the canary pox virus as a viral vector to boost the primary immunization with DNA vaccine. The results were similar to those of the previously discussed study and showed a bright future for the canary pox virus as a viral vector for HCV vaccination[205]. Youn *et al* transdermally immunized 4 chimpanzees with recombinant vaccinia viruses (rVV) expressing HCV genes developed by Marion Perkus at Virogenetics Inc[206]. Upon challenge with infective doses of homologous HCV, 2 control chimpanzees which received only the parental vaccinia virus, developed chronic HCV infections, whereas the immunized animals developed strong gamma interferon-producing T cell responses and moderate T cell proliferative responses[207].

## *HCV-like particles*

HCV virus-like particles (VLP) are safe and easily manufactured vectors for gene delivery that closely resemble the mature HCV structure. Therefore, it is possible to induce neutralizing antibodies and T cell responses against many epitopes using a single VLP-based vaccine. Their usages have been licensed for other viral infections such as the hepatitis B virus (HBV) and human papillomavirus (HPV)[208-210]. Baumert *et al*[211] generated HCV-like particles (HCV-LPs) in insect cells using a recombinant baculovirus containing the HCV structural proteins complementary DNA (cDNA). Structural and antigenic compositions of HCV-LPs were similar to the original HCV, and after testing in mice, it was capable of inducing immune responses against various epitopes of HCV[212]. Another study showed the superior immunogenicity of the HCV-LP-based vaccine compared with the DNA vaccine in transgenic HLA2.1 and BLAB/c mice[213]. This vaccination approach was also tested on a nonhuman primate model - baboons. All 12 animals developed broad and long-lasting HCV-specific humoral and cellular immune responses. Adjuvants only marginally enhanced the immunogenicity of HCV-LP based vaccine[214]. Four chimpanzees were also immunized with HCV-LPs, and they all developed HCV-specific T cell and proliferative lymphocyte responses against core, E1, and E2 proteins. After encountering an infectious HCV genotype, 1 chimpanzee developed transient viremia and the other 3 exhibited higher levels of viremia, but their viral levels became unquantifiable after 10 weeks. Four naive chimpanzees were also challenged with the same HCV genotype, and 3 developed persistent infections[215]. No results of human clinical trials testing HCV-like particles have been published to date.

## *Other strategies*

Gowans *et al*[216] developed a novel peptide delivery system using autologous monocyte-derived dendritic cells. In a phase I dose-escalation clinical trial of HCV patients, dendritic cells were loaded and activated *ex-vivo* with HCV-specific HLA-A2 restricted T cell epitope peptides. All 6 patients who received the vaccine exhibited weak *de novo* HCV-specific CD8+ T cell responses. However, the T cell responses were not continuous, and no change was observed in HCV RNA, anti-HCV antibody, or circulating cytokine levels.

An oral immunization method was introduced using attenuated *Salmonella typhimurium* as a carrier for delivery of HCV DNA to the gastrointestinal tract lymphoid tissue. Vaccination of HLA-A2.1 transgenic mice induced T cell specific responses in 86% of mice that continued for a minimum of 10 mo[217]. Another oral delivery approach was developed using attenuated salmonella carrying a plasmid coding HCV core and E2 proteins. Vaccination of BLAB/c mice induced cellular immune responses and antibodies against HCV core and E2 proteins[218]. Batdelger *et al*[219] evaluated the efficacy of V-5 immunitor tablets, comprising heat-inactivated HCV antigens from HBV and HCV infected donors' blood in 10 chronically infected HCV patients. All of the analyzed patients receiving a daily dose of this therapeutic vaccine tablet showed decreased liver enzymes after 1 month. Moreover, no adverse side effects were observed. However, larger scale and longer studies are required to further evaluate this method and its safety and efficacy.

Plant-based vaccines are another novel approach for developing an easily producible and inexpensive HCV vaccine. The tobacco mosaic virus (TMV) was engineered to encode a chimeric protein containing E2 hyper variable region 1 (HVR1) protein and C-terminal of the B subunit of cholera toxin (CTB). Plants infected with this genetically engineered TMV produced the HVR1 peptide fused to the CTB. HVR-1 specific antibodies acquired from HCV infected individuals reacted with plant-derived HVR1/CTB. Moreover, the intranasal immunization of mice with plant-derived HVR1/CTB chimeric protein induced both anti-CTB and anti-HVR1 serum antibodies[220]. Another attempt at plant-based vaccines used an engineered cucumber mosaic virus (CMV) expressing a HCV 27-amino acid synthetic peptide. Rabbits fed with engineered CMV-infected lettuce plants exhibited an HCV-specific humoral immune response[221].

Chimeric HBV/HCV vaccines are based on the fact that the HBV surface antigen (HBsAg) is a safe VLP-forming delivery system that can carry various antigens including HCV envelope proteins[222]. In 1999, Wu *et al*[223] fused truncated core protein of HCV with the HBV virus and compared its immunogenicity to HCV core protein alone in the sera of HCV-infected patients. The fused antigens exhibited antigenicity to both HBV and HCV, whereas HCV core protein alone resulted in antigenicity to HCV. They tested the chimeric protein in rabbits and mice as well and concluded that chimeric protein is more immunogenic than HCV core protein alone. In another study, HBV core protein (HBc) was fused with HCV core protein (HCc) or HCV non-structural protein 3 (NS3). The purified HBc/HCc and HBc/HCV NS3 were tested in mice. Both induced high humoral and cellular responses to HBV core protein. HBc/HCc led to low antibody and T cell responses to HCV core protein, whereas HBc/HCV NS3 induced higher levels of antibodies and no T cell responses to the NS3 epitope of HCV[224]. Chiron Corp. also invented a chimeric HBV/HCV vaccine using HCV envelope proteins fused with HBV surface antigen (HBsAg)[225]. Another chimeric vaccine was developed by replacing the N-terminal transmembrane domain of HBV surface antigen with the transmembrane domain of HCV E1 or E2 glycoproteins from genotype 1a. The chimeric particles were produced in the stably transduced ovary cells of Chinese hamsters and were used to vaccinate New Zealand rabbits. Vaccination resulted in a strong specific humoral response against both HBV and HCV envelope proteins. Induced antibodies were also able to neutralize HCV pseudo particles derived from heterologous HCV 1a, 1b, 2a, and 3 strains. Furthermore, responses against HBV were equivalent to those induced by the conventional HBV vaccine[226,227]. No clinical trials in chimpanzees or humans have evaluated such chimeric vaccines thus far.

**Conclusion**

This review focused on the development of different types of vaccines against HCV. The arms of the host immune system which are involved in the eradication of the virus were reviewed as well.

Different methods including peptide, recombinant protein, DNA, vector-based, and virus-like particles are currently used to develop and produce HCV vaccines. Peptide vaccines are the leading cause of HCV specific T cell responses. One associated problem is the high rate of genetic variation in viruses within populations and geographic regions. Recombinant protein vaccines are produced by specific gene(s) encoding the immune dominant protein(s). Viral epitopes are the leading cause of immune responses to develop protective immunity, generally including antibodies and CD4+ T cell responses. Vector-based vaccines are manipulated viruses which deliver foreign genetic material to mammalian cells. Viruses can enter cells and express desired genes within the host while becoming non-pathogenic and non-replicative by deleting specific genes. HCV virus-like particles (VLP) are safe and easily manufactured vectors for gene delivery that closely resemble the mature HCV structure.

The challenge for the development of an efficient vaccine with relatively low side effects is still yet to be overcome. Although significant steps have been taken in accomplishing this goal, no promising results have been achieved so far. In part, this depends on the current understanding of the interaction of the virus and the host. Therefore, a more thorough understanding of the host immunity in the context of HCV infection is expected to lead to better results. Of course, other areas of biomedical sciences can greatly contribute to these studies. For instance, providing a suitable animal model for preliminary studies of the newly designed vaccines is crucial.

An effective vaccine against HCV may need to target another cellular process for HCV entry and release or may need to target another sequence of the virus genome. However, due to the high HCV mutation rate, that is not necessarily the best option. A balance between its efficiency in targeting a sequence which can inhibit further growth and a sequence which is highly conserved among different HCV genomes must be found. Alternatively, an efficient vaccine may target several cellular and molecular processes simultaneously. Vaccines may also be accompanied by different adjuvants to increase their efficiency rates. Moreover, other approaches may rely on using other targets, *e.g.*, targeting mRNAs by using synthetic interfering RNAs such as miRNAs and siRNAs.

In addition to attempts to develop vaccines, integrated programs should be carried out to study their effects in individuals; for instance, in which intervals of injection (in case of several infections), in which age group or sex, with which genetic background especially regarding HLA types and genotype variations in different positions of cytokine genes, is it more efficient.

Another seemingly important fact is the relative danger of exposure to HCV or its related particles during studies. This requires laboratories with high safety levels which may not be readily available everywhere, and this can reduce the pace of studies on HCV. It is expected that ongoing studies will eventually lead to the development of more reliable vaccine types, and this in turn would reduce worldwide incidence and prevalence rates of HCV infection.

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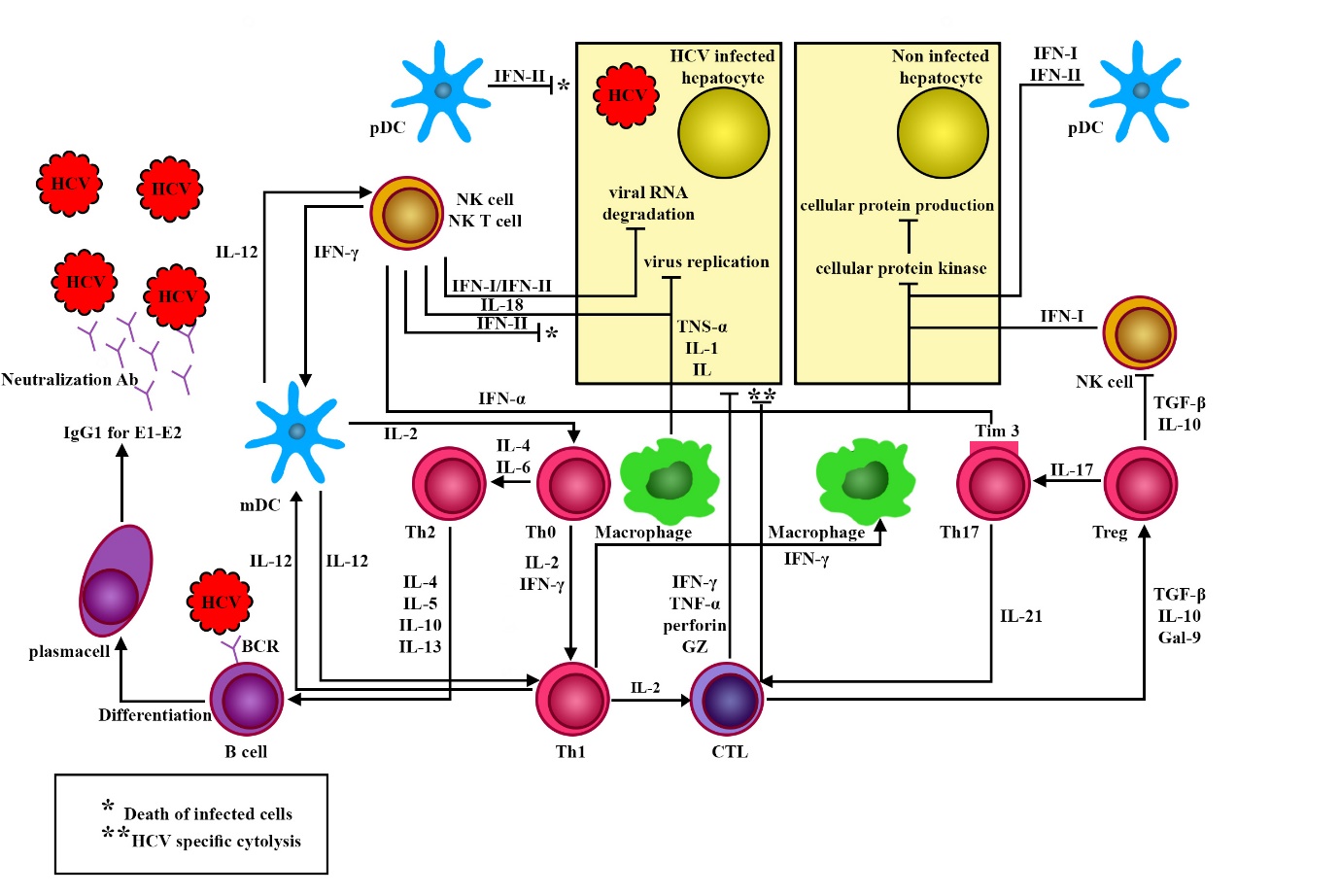
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**Figure 1 Summary of immune responses against hepatitis C virus.** In innate immune response, NK cells are activated which leads to the production of INFs 1-11. This would inhibit the production of viral particles and destruction of their genomes. In addition, in normal cells, antiviral effects are mediated through inhibition of protein synthesis. Adaptive immune responses work through the activation of T cells and production of cytokines that would result in inhibition of the growth of the virus and elimination of the infected cells. B cells, also, perform their actions through the production of neutralizing antibodies against the virus. Ab: antibody; B cell: B lymphocyte; BCR: B cell receptor; CTL: cytotoxic T lymphocyte; E1: HCV E1 glycoprotein; E2: HCV E2 glycoprotein; GZ: [granzyme](https://www.google.com/url?url=https://en.wikipedia.org/wiki/Granzyme&rct=j&frm=1&q=&esrc=s&sa=U&ved=0CBQQFjAAahUKEwjNma_QlN_GAhXwINsKHblkDTU&usg=AFQjCNET86i9WHSGZI2vbvoytFgLcnb-QA); HCV: hepatitis C virus; IL: Interleukin; IFN: Interferon; mDC: [myeloid dendritic cell](https://www.google.com/search?q=myeloid+dendritic+cell&hl=en-IR&biw=&bih=&gbv=2&nfpr=&spell=1&sa=X&as_q=&ved=0CBEQvwVqFQoTCJjwmZyU38YCFSYq2wodSTAO5A); NK cell: natural killer cell; pDC: plasmoid dendritic cell; T cell: T lymphocyte; Th: T helper cell; TGF: [transforming growth factor](https://www.google.com/search?q=TGF:tissue+growth+hurmain&hl=en-IR&biw=&bih=&gbv=2&nfpr=&spell=1&sa=X&as_q=&ved=0CBEQvwVqFQoTCMebytaU38YCFcFp2wod0OsFiw); TNF: tumor necrosis factor.

**Table 1 hepatitis C virus theraputic and preventive vaccines tested in primates and humans**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Type of vaccine | Lead author | Year | Vaccine | Tested in | Adjuvant | Ref. |
| Recombinant protein | Leroux-Roels | 2004 | Recombinant E1  (T2S-918/InnoVac-C) | Human *n =* 20 | Alum | [160] |
|  | Choo | 1994 | Recombinant E1 or E2 | Chimpanzee *n =* 7 | MF59 | [153] |
|  | Frey | 2010 | Recombinant E1 or E2 | Human *n =* 60 | MF59 | [161] |
|  | Drane | 2009 | Recombinant Core | Human *n =* 30 | ISCOMATRIX | [163] |
|  | Verstrepen | 2011 | Recombinant E1 or E2 | Chimpanzee *n =* 4 | Alum | [162] |
|  | Puig | 2004 | Recombinant E1 or E2 | Chimpanzee *n =* 2 | oil/water | [155] |
| Peptide | Firbas | 2006 | 7 HLA-A2 restricted  peptides (IC41) | Human *n =* 128 | Poly-L-arginine | [149] |
|  | Firbas | 2010 | 7 HLA-A2 restricted  peptides (IC41) | Human *n =* 54 | Poly-L-arginine | [150] |
|  | Yutani | 2007 | Peptide vaccine targeting E1, E2, NS3 and NS5A. | Human *n =* 12 | - | [145] |
|  | Klade | 2008 | Synthetic peptide vaccine (core, NS3, NS4) (IC41) | Human *n =* 60 | Poly-L-arginine. | [146] |
| DNA vaccine | Forns | 2000 | DNA vaccine expressing E2 protein | Chimpanzee *n =* 2 | - | [227] |
|  | Sallberg | 2009 | NS3/4A expressing plasmid (ChronVac-C) | Human *n =* 12 | - | [179] |
|  | Alvarez-Lajonchere | 2009 | Plasmid (CIGB-230) expressing core, E1, E2 plus recombinant core protein | Human *n =* 15 | - | [175] |
| Virally vectored | Rollier | 2007 | DNA/MVA | Chimpanzee *n =* 4 | - | [201] |
|  | Folgori | 2006 | Ad6/Ad24 +  electroporated DNA | Chimpanzee *n =* 5 | - | [191] |
|  | Fattori | 2006 | Ad6/Ad6/ChAd32 | Rhesus macaque *n =* 3 | - | [194] |
|  | Barnes | 2012 | Ad6/ChAd3 | Human *n =* 30 | - | [193] |
|  | Youn | 2008 | Recombinant vaccinia | Chimpanzee *n =* 4 | - | [207] |
|  | Habersetzer | 2009 | Modiﬁed vaccinia Ankara virus expressing HCV NS3-NS5B (TG4040) | Human *n =* 15 | - | [203] |
| Others | Elmowalid | 2007 | Virus Like Particles | Chimpanzee *n =* 4 | AS01B | [215] |
|  | Batdelgar | 2008 | V-5 Immunitor–heat- inactivated HCV antigens from HCV infected donors (tablet administered orally) | Human *n =* 10 | - | 219 |

HCV: hepatitis C virus; E1: HCV E1 glycoprotein; E2: HCV E2 glycoprotein.