Infection, Genetics and Evolution 75 (2019) 103968

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Review

Nucleic acid vaccines for hepatitis B and C virus

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ARTICLE INFO

Keywords:
Hepatitis B virus
DNA vaccine
HBV
HCV

ABSTRACT

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections accounts for an important global health problem affecting over 250 million people all around the world. They can cause acute, transient and chronic infections in the human liver. Chronic infection of liver can lead to its failure or cancer. To deal with this problem, alternative approaches or strategies to inhibit these infections have already been started. DNA and mRNA-based vaccination will increase the efficacy and reduce toxicity in patients with Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections. Gene vaccines represent a promising alternative to conventional vaccine approaches because of their high potency, capacity for rapid development, low-cost manufacture and safe administration. MRNA-based vaccination is a method to elicit potent antigen-specific humoral and cell-mediated immune responses with a superior safety profile compared with DNA vaccines. Exploring the intricacies of these pathways can potentially help the researchers to explore newer vaccines. In this study, DNA and mRNA-based vaccination are introduced as an approach to treat Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections. DNA and mRNA-based vaccines as one of the most successful therapeutics are introduced and the clinical outcomes of their exploitation are explained.

1. Introduction

Chronic infection with hepatitis B virus (HBV) is a causative agent of liver cirrhosis or hepatocellular carcinoma (Lee, 1997). They are associated with chronic viral hepatitis worldwide. It is estimated that 30% of the world's population are infected with the HBV virus (Trepo et al., 2014). The transmission of hepatitis B virus emerges via the blood and semen. In endemic areas, the main transmission route is from infected mother to child, while in non-endemic areas, sexual transmission is more common. Transmission can also happen through blood transfusion, dialysis, infectious, and dentistry. Endemic areas infected with hepatitis B virus include China, Southeast Asia, most of Africa, the Pacific Islands, parts of Central Asia and the Amazon forest(Trepo et al., 2014). According to the World Health Organization (WHO), around 3% of the world's population is infected with HCV. About 170 million people are also chronic carriers of this viral infection. Among the chronic carriers, 5–10% of people are at risk of cirrhosis of the liver and carcinoma of the liver cells, resulting in an annual loss of 350,000 people. Liver carcinoma is caused by mechanisms that include chronic inflammation, fibrosis, and oxidative stress following inadequate immune responses to eliminate the viral agent in the body. The infection with HCV, followed by liver failure, has made the virus to be considered as the most important cause of liver transplantation in Western countries (Lau et al., 1997; Yao et al., 1999). The HCV is transmitted through blood transfusion, contaminated medical and dentalequipment, as well as shared needles. The HCV is prevalent in many areas of North Africa, especially Egypt, as well as some Asian countries such as Pakistan, Afghanistan, and China (Ip et al., 2012).

HCV and HBV are among the life-threatening infection which means that exploring new strategies for its treatment is of vital significance. DNA and mRNA-based vaccines have demonstrated high efficiency in the elimination of the disease. This study is an endeavor to review variety of DNA and mRNA-based vaccines developed for HCV and HBV throughout clinical trials.

2. Overview of HBV and HCV virus structure

The HBV virus has a diameter of about 42 nm, including its outer cover. The main protein of the virus called HBsAg, may be attached to the surface domains of preS1 and preS2, producing surface antigens of small HBsAg (SHBs), middle HBsAg (MHBs), and large HBsAg (LHBs). These antigens alone can form virus- like particles (VLPs). The genome of the virus is DNA (Coppola et al., 2015), one of which is a string (negative thread), but has a gap at one end and the other (positive strand) is incomplete. These two strands of the genome of the virus have a length of approximately 3200 open (Gerlich, 2013; Chang et al.,

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https://doi.org/10.1016/j.meegid.2019.103968
Received 2 January 2019; Received in revised form 25 May 2019; Accepted 15 July 2019
Available online 17 July 2019
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2014).

The hepatitis C virus, a small virulent (Envelope) virus belonging to the Flaviviridae family, has a genome of 9.9 kb of RNA. This genome only contains an ORF that codes for a protein of 3000 amino acids. This polyprotein is broken down by protease of the host or by the virus itself into 10 proteins, including structural proteins (which contain central nucleic acid receptor protein (C), E1 coating glycoprotein (gpE1) and E2 glycoprotein (gpE2)), a small membrane polypeptide called P7 and six non-structural proteins (nsPs) including NS2, NS3, NS4A, NS4B, NS5A, and NS5B(Ip et al., 2012).

Hepatitis C virus is introduced into the cytoplasm of cells (liver cells) by binding to specific cell surface receptors including occludin, claudin-1, tetraspanin CD81 proteins and Scavenger class I-type B receptors. After releasing the genome of the virus, which is an RNA virus, viral proteins are expressed. In order to replicate the virus in the host, the genome is multiplied by the RNA-dependent RNA polymerase; a non-structural protein called NS5B. This enzyme lacks error correction and is therefore susceptible to error (Error-Prone). On average, in each episode of viral genome replication, one error occurs. Due to the low half-life and high replication potential of the virus, a large number of similar virus genotypes appearin an individual infected with a virus. Most of the gene changes are associated with glycoprotein genes encoding E1 and E2. Depending on the rate of mutation in the genome caused by the enzyme, it is estimated that HCV is about 10 times more variable than HIV. As a result of the act of this enzyme, there are seven different genotypes of the virus, with over 30% difference due to their nucleotide (Ip et al., 2012; Man John Law et al., 2013).

3. Viral infection: acute and chronic diseases

viral infections are manifested in both acute and chronic phases. In the acute phase of infection, the number of viruses in the blood increases which can be amplified by the PCR method to be detectable. The level of HBs and HBc antigens can be detected by serological methods (Trepo et al., 2014).

After 2–3 months, anti-HBc antibody (of the type IgG) can indicate the active presence of the virus in the patient's body. As the immune system fights the infection to eliminate it, IgM levels decrease and IgG increase. After several months, the level of IgM is also decreased and the IgG antibody increased. The HBs antigen is the main indicator of infection with HBV.

In the chronic phase of the disease, the amount of antigens in the blood decreases. In this phase, the level of IgG against HBc antigen is high, and also the relatively high level of HBs antigen is still measurable. In the chronic phase, due to the infection of liver cells, high levels of alanine transferase (ALT) are also observed (Gerlich, 2013). Because of the non-cytopathic effect of HBV, innate immune responses cannot be generated during the initial phases of infection (Sato et al., 2015).

Almost 5% of infected adults fail to deal with viral replicates, thus, leading to chronic infections indicating that age is a critical contributing factor. Majority of infant populations (90%) progress from acute to chronic infections they age. However, when exposure occurs between one -four year of age, roughly 30% of children show symptoms of chronic disease (Stanaway et al., 2016).

In the chronic phase, inherent immune cells include natural killer cells (NK), liver Kupffer cells (KC), and antigen-presenting cells (APCs), excluding $CD4^+$ and $CD8^+$ T lymphocytes in patients with functional impairment. Meanwhile, antibody responses that depend on T-lymphocytes are disrupted, indicating that anti-HBsAg antibodies have a low-molecular-weight gain for antigen with fewer adverse reactions. In the chronic form of the disease, the virus enters liver cells leading to gradual cellular impairment and cirrhosis of the liver or carcinoma of the liver cells (Chang et al., 2014; Tan et al., 2015).

Like HBV, infection with HCV is also seen in both acute and chronic phases. In 20% of cases, HCV causes an acute infection. The immune response in this phase is induced by neutralizing antibodies and T-cells. Alteration of Alanine Transferase (ALT) has been reported following infection, indicating damage to liver cells. But in the chronic phase of infection, the virus is seen as an immune complex with antibodies without any effective response. Also, T cells immune response seems to be defunct. Continuing this process in the chronic phase of the infection can lead to cirrhosis of the liver and ultimately hepatocellular carcinoma. In the chronic phase of the infection, ALT levels increase. Diagnostic methods of this disease include HCV antibody, ELISA, Western Blot test, HCV RNA and PCR (Ip et al., 2012; Man John Law et al., 2013).

4. Treatment

There are several ways to prevent or treat an HBV infection. The use of nucleotide analogs and antiviral drugs such as lamivudine is one of the commonly used therapies. In the therapeutic approach, called “direct cure”, therapeutic agents directly target the virus to prevent viral replication or expression of viral genes. The response to this therapeutic approach is fairly rapid, but in the established form of the disease, there is a need for long-term use of these agents. The safety of therapeutic agents and drugs are conceived the major issues.

On the other hand, the development of drug resistance is another problem with long-term treatment plan. The high cost of medications call for exploring further therapeutic approaches. A treatment so-called “indirect treatment” is based on the activation of the immune system against the viral infection which is carried out either via active or passive techniques. Disabling immunization is based on the use of the cytokines, antibodies or pre-activated cell lines against the virus. In this type of immunization, an appropriate response to the hemorrhagic and cellular immune system is formed against the virus; however, there is a need for frequent immunization. In addition, the chances for the absolute elimination of viral infection are rare. In active immunization, the inherent immune response to viral infection is stimulated. Active immunization is relatively long-lasting, and if used at appropriate intervals, it will have a good effect. At present, the use of antiviral drug lamivudine with interferon alpha as the standard treatment method (SOC) is considered for viral infection of HBV (Noordeen, 2015; Wen et al., 2014).

Immune defense system deficiency of host to remove HBV during acute infection leads to viral durability and chronic infection. Infection of chronic HBV is progressive and patients are found to undergo five different phases of disease as abridged by Spearman and co-workers in 2013 (Spearman et al., 2013).

New generation of antiviral such as tenofovir and entecavir, are not drug-resistantas lamivudine or adefovir. However, the main drawback of the antiviral treatment for chronic hepatitis B is that it is not curative and has to be taken life-long and only delays onset of the disease (Hosaka et al., 2013).

The standard treatment for HCV disease is the weekly administration of PEG ylated interferon alfa with a regimen of an antiviral combination of ribavirin twice daily for 24 to 48 weeks. This long-term treatment is associated with side effects such as flu-like symptoms, depression, and anemia. In addition, these drugs are effective in less than half of the people with genotype 1 virus (and 80% of patients with genotype 2). A further challenge in terms of antiviral drugs is their being expensive. Which has led to speculation upon generation of therapeutic or preventive vaccines; though, there are fundamental obstacles in the development of these vaccines against HCV infection which include:1)outstanding genetic variation in the genome of viruses caused by the function of the NS5B enzyme, which is an RNA polymerase susceptible to error. 2) various mechanisms for the escape of the virus from the immune system, which causes chronic infection to persist in the body. 3) the lack of suitable animal models for infection with HCV. In general, chimpanzees and a small mammal called Tupaia Belanger are the natural hosts for this virus, although maintenance and handling of these animals are demanding and costly. In addition, there

are differences between the infection with HCV in humans and chimpanzees; For example, in humans, about 85% of cases of HCV infection are related to the chronic phase, while in chimpanzees this amount is 30–40%. Unavailability of animals and the ethical issues are among limitations inusing animal models (Man John Law et al., 2013; Ghasemi et al., 2015).

In CHB, because of the high rates of the viral replication and unabated antigen exposure, an exhausted CD-8+ T cell phenotype can be characterized by the loss of IFN-γ production in response to the stimulation with antigen (Guidotti et al., 2015). Exhausted memory CD8+ T cells do not produce IL-2, and lose their high proliferative activity [19]and no longer have the capacity to kill during initial stages of the conversion to the exhausted phenotype (Wherry, 2011). Finally, T cells become dysfunctional and no longer can produce TNF-α and IFN-γ which results in cytotoxic functioning inhibition. At last step, this process leads to antigen-specific T-cell populations loss. The chronic viral infection can affect CD-4+ T cell populations (Brooks et al., 2005), although the exact underlying mechanism remains to be explored. Some studies indicate that dysfunctioning of CD-4+ T cell lead to a decrease in the production of a cytokine which contributes to maintaining antiviral CD-8+ populations called IL-21R (Fröhlich et al., 2009). Other studies demonstrate that an increase in the level of IL-10 can be expounded by dysfunctioning of CD4+ T cell (Brooks et al., 2006).

In CHB, numerous viral and host-dependent elements aid in the establishment of exhausted HBV-specific T cells and reduction of anti-HBV immunity. Continuous exposure to HBcAg, HBeAg, and HBsAg in the absence of the suitable secondary signals for activation of T-cell can result in T-cell anergy in patients with CHB (Reignat et al., 2002). In patients with chronic infection liver, tolerance can be promoted by high amounts of IL-10 and transforming growth factor-beta (TGF-β) instead of stimulation of the immune response against HBV. Inhibitory programmed death protein 1 (PD-1) overexpression on hepatocytes, can enhance the synthesis of mucin domain 3 (TIM-3), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and T-cell immunoglobulin, which all act to suppress the activity of the immune system and also increase tolerance (Guidotti et al., 2015; Peppa et al., 2010). Moreover, regulatory T cells (Tregs) and IFN-γ-deficient NK cells cause a failure in the response of the CD-8+ T-cell to antigens of HBV (Isogawa et al., 2013).

Due to the substantial cost and risk of numerous serious side-effects, IFN-based treatments havebeen roughly failed (Rehermann and Bertoletti, 2015). The first line of treatment can reduce the viral load as checked by anti-HBsAg seroconversion, and decrease the risk of complicating disease (Hosaka et al., 2013). However, seroconversion alone is not indicative of curative therapy. Due to the existence of cccDNA, disease relapse has been reported in most HBV infections (Zoulim and Locarnini, 2009).

cccDNA transcription can be controlled by four various promoters and two enhancer elements and the transcription of a gene regulated similar to gene transcription in host cells, via numerous transcription factors, co-activators, enzymes, and corepressors which play a significant role in the chromatin modification (Peterlin et al., 2018; Tropberger et al., 2015). cccDNA is stable and has excellent durability, at the same time, the immune system cannot make any impressive responses in opposition to HBV (Allweiss et al., 2017; Bloom et al., 2018; Werle–Lapostolle et al., 2004). Accompanied by delivery of siRNA approaches to decline the levels of cccDNA and as a result, control of HBV infections, there is another important strategy to achieve complete cure as a consequence of eliminating cccDNA(Jiang et al., 2017; Lin et al., 2014; Moyo et al., 2018).

There are many strategies for the treatment of HBV infections which involve the use of gene editing technologies such as TALENs or RNA interference technology (RNAi) (Ivacik et al., 2011; Bloom et al., 2013) to destroy viral genomic material and interrupt the expression of a viral gene, which respectively have become prevalent approaches of HBV genome silencing.

Recently, anti-HBV vaccination is carried out in more than 180 countries in the world and has been accredited with decreasing the rate of chronic carriers in the world by 70–90% (Gerlich, 2015).

5. Therapeutic vaccines for HBV

Acute HBV infection removal needs both strong cellular and humoral immune responses wherein CD-4+ and CD-8+ cells have significant roles in the eradication of infected hepatocytes and viral particles. A tolerogenic hepatic microenvironment characteristic of an exhausted T-cell phenotype can instigatefrom CHB (Isogawa et al., 2013). After resolution of acute infection, CD-8+ T cells produce extremely functional populations, memory T cells with special distinctive attributes that differentiate them from their antigen-naïve, memory-like T cell counterparts. Properties of memory CD-8+ T cells include (i) fast re-activation after antigen re-encounter, (ii) great proliferative capacity after re-activation, (iii) homing to secondary lymphoid tissues and (iv) protracted durability via the population homeostatic maintenance through interleukin 7 (IL-7)- and IL-15-driven proliferation (Virgin et al., 2009).

Nucleic acid vaccination methods, thus, have great potential not only for prophylactic but also for the immunotherapeutic benefit. Substantially, repeated vaccination with DNA Hepatitis B core antigen (HBsAg), encoding preS1/preS2/S proteins, Polymerase (Pol), interleukin (IL)-12 adjuvant, and HBx can elicit potent anti-HBV cell and humoral-mediated immunity (Yang et al., 2006). Electroporation cannot obtain the highestefficiency from in situ encoded antigens because of the need to specific delivery to APCs. The immunogenic effect of the vaccine can be considerably improved by using recombinant protein to DC-cells targeted through DNA vaccine (Wang et al., 2016) and in non-target tissues, decline the risk of aberrant immune stimulation.

Therapeutic vaccination method should induce not only humoral but also cellular immunity with straight antiviral effect (Shouval et al., 2015).

There are some challenges including the optimal and successful delivery of DNA sequences to targeted tissues and DNA vaccine studies often yield conflicting and extremely variable efficiency data. The variability of results from studies of DNA vaccination and previous safety concerns have raised concerns about using DNA-based treatment with human patients. The use of DNA-based vaccination approaches for HBV steel is in its primary stages. DNA vaccines even in combination with classical nucleos(t)ide analogue (NUC) treatment, still have to go a long way to be favored over other immune-therapies. Significant DNA vaccines in clinical trials listed in Table 1 forseveral types of cancer and Hepatitis B virus and Hepatitis C virus infections (Yoon et al., 2015). mRNA-based vaccination is a method by which to induce potent antigen-specific humoral and cell-mediated immune responses. mRNA vaccines have many advantages including significant safety, achieving high efficacy by formulating mRNA, prompt, inexpensive, and vast production.

Formerly, mRNA vaccines for stimulation of CTL immune responses were designed as a promising method for cancer immunotherapy or for HIV therapy (Boudreau et al., 2011; Vanham and Van Gulck, 2012). mRNA immunotherapy was carried out by isolating DCs from a patient and manipulating them ex vivo with the mRNA construct and then reintroduced to the patient. In spite of clinical feasibility and safety, ex vivo DCs engineering is a form of personalized medicine which is expensive and intricate. Aforementioned challenges significantly restrict its application within the common population (Vanham and Van Gulck, 2012) (see Table 2).

The present study was intended to introduce an anti-HBV mRNA vaccine in order to improve prophylactic strength via the inclusion of all required HBsAg epitopes and also explore the application of mRNA vaccination as an innovative immunotherapeutic (Lamb, 2017).

There are currently four types of therapeutic vaccines against

Table 1
Significant DNA Vaccines in clinical trials.

DNA vaccines	Delivery method	Diseased condition	Phase	Status	Pharmaceutical factories	Identifiers from Clinical Trials
pGA2/JS2	Intramuscular (IM) injection	HIV	I	Completed	National Institute of Allergy and Infectious Diseases (NIAID)	NCT00043511
VCL-IPT1andVCL-IPM1	IM injection	Pandemic Influenza	I	Completed	Vical	NCT00709800
VGX-3100	IM injection and Electroporation	cervical dysplasia (high-grade squamous intraepithelial lesions)	III	Recruiting	Inovio Pharmaceuticals	NCT03185013
INO-3112	IM injection and Electroporation	Cervical Cancer	II	Completed	Inovio Pharmaceuticals	NCT02172911
mRNA vacciene CV9103	Intradermal (ID) injection	Hormonal Refractory Prostate Cancer	II	Completed	CureVac AG	NCT00831467
CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine	ID injection	Patients With HER2-Negative Stage III-IV Breast Cancer	I	Recruiting	University of Washington	NCT02157051
Neoantigen DNA Vaccine	IM injection and electroporation	Pancreatic Cancer Patients	I	Recruiting	Washington University School of Medicine	NCT03122106
pVAXrcPSAv53l	ID injection and Electroporation	Prostate Cancer	II	Completed	Uppsala University	NCT00859729
pTVG-HP	ID injection	Non-metastatic Prostate Cancer	II	Active, not recruiting	University of Wisconsin, Madison	NCT01341652
VRC-EBODNA023-00-VP and VRC-MARDNA025-00-VP	IM injection	Ebola and Marburg Virus	I	Completed	National Institute of Allergy and Infectious Diseases (NIAID)	NCT00605514
INO-4212 and With or Without INO-9012	ID or IM injection followed by Electroporation	Ebola	I	Completed	Inovio Pharmaceuticals	NCT02464670
GLS-5700	EP-enhanced intradermal (ID) injection	Dengue Virus Seropositive Adults	I	Active, not recruiting	GeneOne Life Science, Inc.	NCT02887482
pCMVS2·S	IM injection	Chronic B Hepatitis	II	Completed	French National Agency for Research on AIDS and Viral Hepatitis	NCT00536627
VRC 705	IM injection	Healthy Adults and Adolescents (Prevention)	II	Recruiting	NIAID	NCT03110770

Table 2
Overview of significant gene vaccines in Hepatitis B and Hepatitis C.

Drug name	Class	Phase	Disease	Company	Identifiers from Clinical Trials
DV-601	Therapeutic vaccine: viral antigen complex based	Ib	Hepatitis B	Dynavax	NCT01023230
Hi-8 HBV	Therapeutic vaccine: viral antigen complex based	II	Hepatitis B	Oxxon	
ePA-44	Therapeutic vaccine: viral antigen complex based	II	Hepatitis B	Chongqing Jiachen Biotechnology Ltd	NCT00869778NCT01326546
HB-110	Therapeutic vaccine: DNA based	I	Hepatitis B	Genexine	
HBV-DNA plasmid pdpSC18 vaccine	Therapeutic vaccine: DNA based	I	Hepatitis B	PowderMed/Pfizer	
GS-4774	Engineered to express a fusion protein containing HBsAg sequences	II	Hepatitis B	Gilead Sciences, Inc	NCT02174276
TG1050	Therapeutic vaccine: Adenovirus based	I/Ia	Hepatitis B	Transgene	NCT02428400
INO-8000	NS3, ،NS4A,،NS4B, NS5A	I/ IIa	Hepatitis C	GeneOne Life Science; Inovio Pharmaceuticals; Mayo Clinic	NCT02772003

hepatitis B virus infection that undergo various stages of clinical trials including protein/peptide vaccines, antigen-antibody-based vaccines, cell-based vaccines. In peptide/protein vaccines, anti-HBs and HBc antigens or derivative peptides stimulate the immune system. Inimmunizations with the antigen-antibody vaccine, the antigen presenting cells are activated with aself-pacing complex activatingantigen-specific T cells. The production of antiviral cytokines increase interferon. In cell-based vaccines, mainly intrinsic immune cells, including NK cells, or most commonly antigen-presenting dendritic cells that have been activated in vitro with viral peptides or viral genes, are used. In genetic vaccines, nucleotide sequences encoding viral antigens or part the sequence are used in a plasmid vector and are injected using electroporation technique (Wen et al., 2014). Plasmids used in the preparation of genetic vaccines contain nucleotide sequences that express HBsAg and HBsAg proteins. These gene vaccines have been implemented in numerous pre-clinical studies. The animal models used in these studies have been transgenic mice or an inventory called Woodchuck, which hosts the virus naturally (Lu et al., 2007; Kosinska et al., 2015; Dembek and Protzer, 2015).

A DNA vaccine encoding a protein sequence of HBV viral infection has also been used in primates. It has been shown that the vaccine produces immune protection by inducing a cellular immune response (and not humoral immunity) in chimpanzees. Protein antibodies were produced when the virus-encoding protein similar to the HBV virus was used as a reminder, and the level of the virus in the plasma was unidentified for 6 months (Mancini-Bourgine and Michel, 2005).

The first clinical trial of the vaccine against HBV was carried out by Maryline Mancini-Bourginein France and the results were published in 2004 (Mancini-Bourgine et al., 2004). The vaccine was a gene sequence which was coded for the medium and small HBV viral proteins in the pCMV plasmid(Mancini-Bourgine et al., 2006). In the first study, 10 patients with chronic HBV infection who did not respond to standard drugs were selected and were chronically infected for a long time, i.e., as carriers of the virus. Patients received intramuscular injection (i.m) of deltoid muscle three times a day (0.5 mg dose) with two-month intervals. The vaccine was injected into six patients within 10 months. Ultimately, T-cells responding to HBV viral antigens were evaluated using the ELISpot technique. The results showed that after the third injection, two patients whose blood contained T-cells responded to viral antigens. Also, after the third injection of the vaccine, the response rate of the specific T-cells that produce interferon-gamma also increased significantly (Fig. 1). A quantitative PCR analysis pointed out that the serum level of the virus reduced after the third injection of the vaccine in five patients in a way that in one patient, viral infection was not observed in the serum. Consequently, the results of this clinical trial showed that the designed vaccine was safe and also effective in inducing an immune response against viral infection (Mancini-Bourgine et al., 2004).

Another clinical trial in this group was conducted in patients with chronic viral infection who responded effectively to analogs. In this clinical trial, the phases I / II, was performed on 59 patients divided into two groups. While one group did not receive any vaccine, while the other group comprising 31 patients who received the vaccine. All patients were effectively treated with analogs and at the beginning of the study, their serum contained interferon-gamma secreting T-cells against viral antigens. The aim of this study was to investigate the potential positive effects of the vaccine after treatment with analogs. The results showed that in the group receiving the vaccine, the level of viral antigen-specific T-cells remained stable in the body of the patients. The control group did not receive the vaccine and the level of T-cell responders declined over time (Godon et al., 2014).

In another clinical trial, a binary DNA vaccine was used against chronic HBV infection. In this study, 39 patients with chronic HBV infection were selected and were divided into three groups. The first group included individuals treated with the vaccine whose serum level of alanine transferase (ALT) was approximately one to two times than the highest normal range. 33 patients with rather higher serum level were assigned to two groups in a 2:1 ratio: The group that received both Lamivudine (LAM) and the vaccine (Group II) and the group that received only lamivudine (Group III) (Yang et al., 2012). In this study, two plasmids were used. One of the plasmids contained the MHBs antigen-encoding gene and the other was an adjuvant plasmid and contained the interleukin-2 fusion protein and interferon gamma encoding gene. Vaccination was also performed at 0, 4, 12 and 24 weeks (Yang et al., 2012). The results of the study showed that in the first group, the number of T cells secreting viral specific gamma-interferon increased after receiving the vaccine. The results indicated a decrease in the genotoxicity of the HBV virus in the serum of the subjects in both second and third groups. The decrease was more evident in the second group, which received both the vaccine and the medicine. Finally, the results of this study showed that the use of binary DNA vaccine, when used concurrently with Lamivudine, is more effective than the medicine alone. However, there were no severe side effects in the subjects studied (Yang et al., 2012). Also, mild side effects were not attributed to vaccination. However, the continuation of this study showed that the vaccine does not prevent the recurrence of chronic viral infection seen in most treated patients (Fontaine et al., 2014).

A new clinical trial was conducted in South Korea using the HB-110 vaccine. This clinical trial (Identifier: NCT00513968) was performed on 27 patients with chronic viral infection. The subjects were divided into two groups: the group receiving ADV alone, an antiviral nucleotide analog, and the group that received ADV and the vaccine. The results of this study showed that a different dose of the HB-110 vaccine does not cause any specific side effects in patients. Furthermore, the vaccine was associated with lowering and normalizing the serum ALT level (Yoon et al., 2015).

6. Therapeutic and preventive vaccines for HCV

In general, vaccines against HCV are divided into two broad categories of therapeutic and prophylactic vaccines and have been used to re-activate the virus-specific T cells that have lost their function due to infection. These cells release the antiviral cytokines after activation or migrate to the liver to destroy the virus-infected cells. Prophylactic vaccines have been designed to induce a host immune response. There are three main approaches to achieve preventive vaccines:

In the first approach, the E1 and E2 glycoproteins of the virus are targeted at inducing neutralizing antibodies against the virus. Most prophylactic vaccines are in this category. The main problem in this approach is the heterozygosity of the HCV virus, which has 7 different genotypes. Most antibodies produced in this approach are specific to the genotype and are only used against a particular genotype of HCV. However, some also detect protected areas throughout the glycoprotein and thus affect several genotypes with the same E1 or E2 glycoprotein structure as their spatial structure. In the second approach, the cellular immune response is the goal of vaccination. In this approach, both humoral immune response and activated cellular immunity can have a synergistic effect on the elimination of infection. Types of peptide-protein vaccines, recombinant viral vaccines, and plasmid DNA vaccines have been used in these two approaches. In the third approach, viruses are killed or viral particles (VLPs) are used. Completely killed viruses are inactive after being propagated in a cell culture medium and used as a vaccine (Man John Law et al., 2013; Liang, 2013). The main problem in producing these vaccines is the low efficiency in the production of the virus. Viral glycoproteins are capable of forming viral-like particles. However, inducing a humoral immune response with the production of neutralizing antibodies is due to the vitality of placing glycoproteins in the particle structure.

In the early 1990s, it was found that a plasmid containing the nucleoprotein (NP) virus encoding the influenza A virus, when injected intramuscularly into animals, can stimulate antibody responses as well as CTLs. This study set the groundwork for the advance of genetic

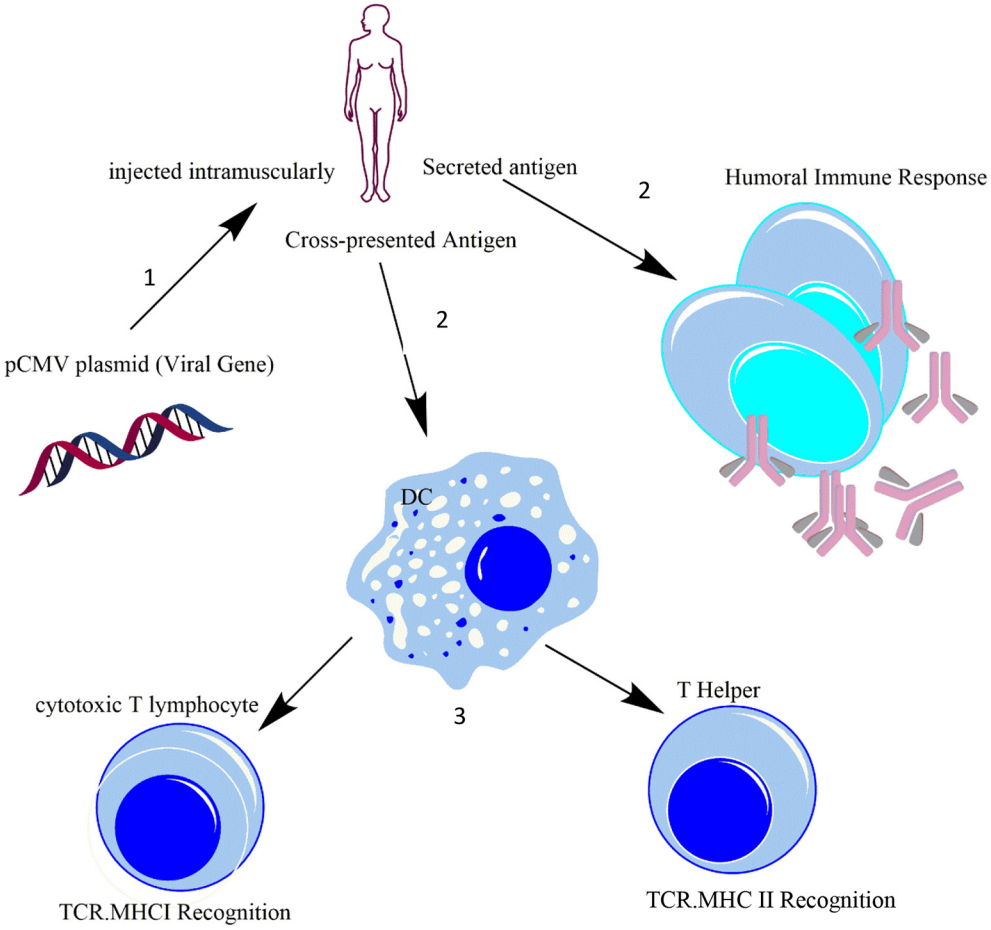

Fig. 1. Mechanism of DNA vaccinesFor HBV. The vaccine used was a gene sequence which coded for the medium and small HBV viral proteins in the pCMV plasmid. Patients were injected intramuscularly (i.m).after immunization, transfected muscle cells may produce antigen that stimulate B cells of the immune system, which they produce antibodies.Transfected muscle cells could transfer the antigen to antigen presenting cells such as dendritic cells. They processing and display of MHC-antigen complexes arise.

vaccines against infectious agents along with certain types of cancers (Chen et al., 2011).

Plasmids that comprise sequences encoding a number of proteins or epitopes of the HCV virus are capable of inducing cellular and humoral immune responses in animal models when used intramuscularly (i.m) or subcutaneously (s.c). It has been determined that the use of plasmids containing structural or non-structural genes of hepatitis C virus in animal models can stimulate the response of viral T-specific cells. For example, Chen et al. (2011) used a gene-containing plasmid containing central C protein in model mice (due to the constant presence of non-structural anti-HCV NS3 / 4 antibodies, the response of their specific T cells to these antigens). The group showed that following vaccine injection, intrinsic activating pathways, and T heterologous lymphocytes were activated, resulting in an environment that was recovered to restore immune response to viral infection (Chen et al., 2011).

It has also been shown that the use of gene cytokines such as IL-2, GM-CSF, INF-α or Flt-3 L when used as an adjuvant with a vaccine, can protect mice against the expression of tumor antigens induced by HCV (Encke et al., 2006). Also, some viral proteins in HCV cause the immune response to be tolerated in the host. When the modified gene of these proteins is used, immune responses are more effective than natural gene of the virus (Zhu et al., 2010; Li et al., 2006).

Appreciating considerable benefits of genetic vaccines, it is anticipated that an imperative step in the treatment of viral infection with hepatitis C be taken if the vaccines are appropriate. Currently, there are two examples of DNA vaccines at different stages of a clinical trial, which will be described in more detail below.

The first DNA therapy vaccine against HCV, which was evaluated in Phase I of the clinical trial, was CIGB-230. The vaccine contains the plasmid pIDKE2, which contains the central protein (C), E1 and E2 expression signal sequence, and is used in conjunction with the recombinant protein of the central protein Co.120. At the stage of the clinical trial, 15 patients, including 7 women and 8 men aged 39–59 years, with an average age of 49 years with chronic viral infection who did not respond to standard treatments (interferon and ribavirin) were selected. This study was conducted at the National Institute of Gastroenterology, Havana, Cuba. Exclusion criteria included pregnancy, nursing, and infection with HIV and HBV (Alvarez-Lajonchere et al., 2009; Castellanos et al., 2010).

The vaccine (CIGB-230) with0.5 mg of pIDKE2 plasmid contained a sequencing of 650–1 amino acids from the polyprotein derived from the Cuban genotype 1b. This sequence is controlled by a strong CMV viral promoter and finally contains the pA termination sequence obtained from the SV40 virus and the polyadenylation sequence (Duenas-Carrera et al., 2002).The vaccine also contains 0.05 mg of the central recombinant HCV protein (sequence 120–1) found in the host *E. coli* and the pSLCol20 plasmid (under the control of the promoter and the T7 termination sequence) (Acosta-Rivero et al., 2005).

In this study, each patient was injected intramuscularly (i.m) six times at intervals of 4 weeks. Side effects such as a headache were observed in some of the subjects, but autoimmune responses including mitochondrial-reactive antibodies and cell nuclei, seen in some DNA vaccines, were not considered. The results of this clinical trial study showed that in 7 patients, the antibody response to the viral infection improved. Also, 24 weeks after the first immunization with the vaccine, 73% of the patients contained T-specific cells and interferon-gamma secreting cells (against central antigens (C), E1, E2, and NS3). The results showed that although only one patient showed a decrease in viral RNA levels in the serum, in 40% of them, liver tissue improved, which mainly included reduction of liver fibrosis, which is the result of the

immune response to the virus during the chronic phase of the disease. The final result was that the vaccine, CIGB-230, is a safe vaccine in humans, but requires different optimizations to improve its efficacy, including vaccine dose and injection method (Amador-Canizares et al., 2014). Following the results of the first clinical trial with this vaccine, another trial (Phase II) study was conducted in patients with a chronic viral infection. In this study, 92 patients were treated with CIBG-230 vaccine plus standard therapies including interferon and ribavirin (Alvarez-Lajonchere et al., 2009; Castellanos et al., 2010).Finally, the results of this study showed that in a group of patients who received the vaccine with interferon and ribavirin, the response of the specific T cells was more active, with greater proliferation and increased interferon secretion, as well as augmented specific viral antibodies. The second vaccine in the clinical trial stage is the ChronVac-C® (ChronTech Pharma AB) vaccine, which includes the plasmid expressing the unstructured NS3 and NS4A proteins derived from the Hepatitis C virus genotype. The vaccine has undergone the Phase I clinical trial and is currently in Phase II trial (Weiland et al., 2013).

In Phase I of the clinical trial, 12 subjects with chronic hepatitis C virus infection (genotype 1) who had not been treated were selected including 7 men and 5 women with mean age of 46 years. HCV RNA levels, T-cell responses to the virus as well as liver fibrosis were measured before and after the study. The vaccine was injected intramuscularly in different doses twice a week with a 4-week interval. An in vivo electroporation technique was used to inject the vaccine using the Inovio'sMedpulser® system. Three patients (167 micrograms), three (500 micrograms) and six (1) 1500 micrograms of DNA were injected. Also, standard treatment with ribavirin and PEGylated interferon was prescribed to some patients. Standard treatment was provided on average 15 months after vaccination (1 to 30 months). During the study, using the ELISpot technique, specific T-cells responding to viral antigens and anti-viral cytokine secreting cells were studied. HCV RNA levels were also measured.

The results of this phase of the clinical trial indicated a significant increase in interferon-gamma expressing cells in response to viral infection during the first 6 weeks of treatment. Also, 5 (41.6%) patient's demonstrated a decrease in HCV RNA levels in the first two to two weeks. These results indicate a temporary immune response to viral infection following vaccination (Hosaka et al., 2013). The study also exhibited that 6 out of 8 (75%) patients receiving standard therapy were treated. As a result, combining vaccine and standard treatment in patients with chronic hepatitis C infection is preferable. However, given the fact that the number of subjects at this stage of the clinical trial is limited (a total of 12), it cannot be proved that the vaccine is safe and also effective at this stage. For this purpose, the Phase II clinical trial is underway with a more patient-focused treatment strategy (Weiland et al., 2013).

Some clinical trials have been designed relying on viral recombinant viruses. In a clinical trial published in 2011, F. Habersetzer et al. implemented a viral vector containing NS3, NS4, and NS5B encoding genes. The clinical trials with this viral vector called TG4040 indicated the safety and efficacy of the vaccine. According to the study, the vaccine was well tolerated in the studied groups without severe side effects. The findings correspondingly disclosed that in 33% of cases (5 out of 15 patients with CHC), immune cellular immunity was achieved 6 months after the first dose of the vaccine. Also, in 8 patients, the level of RNA of the HCV virus significantly decreased (Habersetzer et al., 2011).

In a further clinical trial, Swadling et al., 2014 used adenoviruses of monkies to vaccinate the patients. In a former study by this group, a human adenovirus vector (Ad6) heterologous vector with chondrogenic adenovirus vector (ChAd3) encoding NS3-NS5B and depleted polymerase NS5B was used as a reminder. The results displayed that the use of Ad3 vector can produce a strong cellular immune response. However, two limitations of this study led to an alternative study by this group; first, after the prescription, the level of immune response to the surface did not reach the level after Ad3 injection, and secondly, most of T cells were activated in volunteers of type TCD8 +. Nevertheless, in an ideal vaccine, the activation of both CD4 and CD8 T cells is important (Barnes et al., 2012).

To address the aforementioned limitations, two replication defects in the ChAd3 vector were used and MVA encoding NS3, NS4, NS5A and NS5B (related to Genotype 1b) were measured. Intraviolet imaging (i.m) did not cause any special effects in volunteers, or side effects that occurred after 24 to 48 h disappeared. The results of this study showed that the subjects responded to ChAd3 as well as to MVA as a reminder, which led to the production of specific T cells of HCV antigens. In addition, the results showed that T cells are functional and have both CD4 + and CD8 + type. It was also indicated throughout this study that T-cells activated a variety of HCV viruses with genotypes 1a, 3a, and 4a, with a percentage difference of up to 86% on the protein level of genotype 1b (71Nnnl,;;lolooolo,lnu8P + F). Using pseudo-typed chimpanzee adenoviral vectors encoding non-structural HCV proteins successfully induced robust and potent anti-HCV T cell responses (Kelly et al., 2011).

7. Conclusion

Despite many advances in anti-HBV infection therapy, the failure of current treatment regimens is not bewildering. Part of this failure may pertaining to increasing viral resistance, debilitating adverse side-effects, and more importantly, low efficacy of regimens. Such challenges have impelled many researchers to look for innovative therapeutic strategies for the treatment of HBV infections. It was found that Gene Vaccine can increase the efficacy and reduce toxicity in patients with HBV and HCV infections.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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