Review article

Recent Approach in Prophylactic Vaccine Development against Hepatocellular Carcinoma: Insights from Reverse Vaccinology

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Abstract: Hepatitis C virus (HCV) has chronically infected more than 200 million populations. It is a giant global public-health issue of this era. A significant effort has been made in the field of HCV therapy both in prophylactic and therapeutic strategies. There appears to be a high degree of variation and inconsistency in the understanding of pathophysiology of the disease and its pathogen due to different patterns of study, diagnostics and prediction criteria of the vaccine against the pathogen. However, few studies have so far been reported across the world on the reverse vaccinology approach and peptide vaccine prediction. Nevertheless, more study is required in order to enhance the accuracy and fine-tuning the previously known facts. This review summarizes the risk factors associated with the virus and its complete understanding of HCV linked cancer based on findings from epidemiologic and meta-analyses data.

**Keywords:** HCV; Reverse Vaccinology; HCC; Peptide Vaccine; Epidemiology

1. Introduction

Hepatitis C virus (HCV) is an infectious disease-causing agent. The prevalence rate is nearly 3% of the total population worldwide (Amer 2014). A person with HCV infection sometimes re main asymptomatic or undiagnosed for several years and thus it leads to chronic hepatitis and results into severe fibrosis, cirrhosis (Freeman et al. 2001) and hepatocellular carcinoma (HCC) (Mühlberger et al. 2009), thus requiring liver transplantation in many patients (Mühlberger et al. 2009, Perz et al. 2006) or may cause death of the patient. The genus was identified in 1988. Research work has revealed that the developing countries are at more risk for viral diseases like hepatitis C virus (Hunziker et al. 2001). Recent discovery of direct acting antiviral agents (DAAs) against HCV has made an endeavor for therapeutic advancement (Au and Pockros 2014, Casey and Lee 2013). In addition, challenge remains in employing modern antivirals in patients with asymptomatic HCV infection and targeted result must be achieved through different

strategies. Numerous clinical trials have shown various combinations of agents, including interferon-free regimens, to be favorably effective in the clearance or sustained viral response (SVR) to chronic infection of hepatitis C. HCC ranked the fifth common type of cancer and accounts about ~5.6% of all cancers worldwide (Bosch et al. 2004, Sherman 2010). The cases of HCC are rising colossally. If we do not follow the safety measures from now only then it will take little time to turn into a drastic condition near future. Liver fibrosis is emphatically linked with HCC, on an average 80-90% of HCC cases are emerging in cirrhotic livers (Lok et al. 2009, Seitz and Stickel 2006). HCC development is additionally associated to alcoholic cirrhosis (Fattovich et al. 2004), non-alcoholic steato-hepatitis (NASH) (Ascha et al. 2010).

2. Risk of HCV infection

HCV is a very tiny, circular, encased, single sense-stranded RNA virus and comes under the hepacivirus genus within the Flaviviridae family with a diameter of approximately 50-60nm (Bostan and Mahmood 2010) Fig.1. The genome of the infectious virus is about 9.6 kb in size and it is structured within an ORF that synthesizes a long protein sequence roughly around 3000 amino acids. The protein is translated through multiple sequence of events from the starting of the genome and consequently sliced by cell proteases and viral peptidase into three different types of fibrous proteins *i.e.* Core protein, environmental proteins (E1 and E2) and other non-structural (NS) proteins like are p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Reed and Rice 2000). The processes within the cell of the virus life are closely associated with non-structural (NS) proteins (Grakoui et al. 2003). The N-terminal region of the ORF encodes structural proteins, while the other part of the ORF encodes the nonstructural proteins Fig.2 (Miller and Purcell 1990). HCV is characterized by exceptionally high hereditary variability,which is especially true for envelope proteins responsible for viral entry into the target cell (Smith et al. 2014). HCV has 1-7 distinct genotypes with about 30% genetic variability (Messina et al. 2015). Constant efforts are being made to prevent transmission and to develop chemotherapeutic regimens for this leading public health problem.
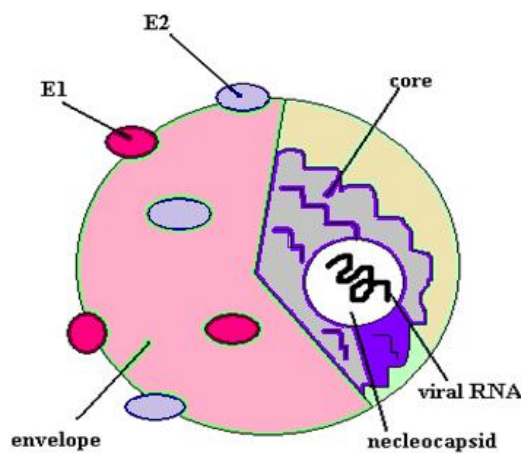
Figure 1. Structure of Hepatitis C Virus

The core protein combines to form the viral nucleocapsid and contains the viral RNA genome Fig.3. The HCV 5'-UTR (untranslated region) contains a 341nt sequence, the main conserved region of the genome, and located upstream of the ORF translation start codon (Choo et al. 1991). The 3'-UTR consists of 225nt approximately where it is arranged into more than two different locales; an extremely variable region (30–40nt), poly(U)-poly(U/UC) tail, and the last one is a very highly conserved 3'-terminal stretch (98nt) *i.e.* 3' X region (Han et al. 1991). The stem-loop structures of 3' X region consists of Stem-Loop 1, Stem-Loop 2 and Stem-Loop 3 (Kolykhalov et al. 1996). Several studies revealed that some of the 3' UTR regions appear to improve the replication of virus (Friebe and Bartenschlager 2002, Ito and Lai 1997).
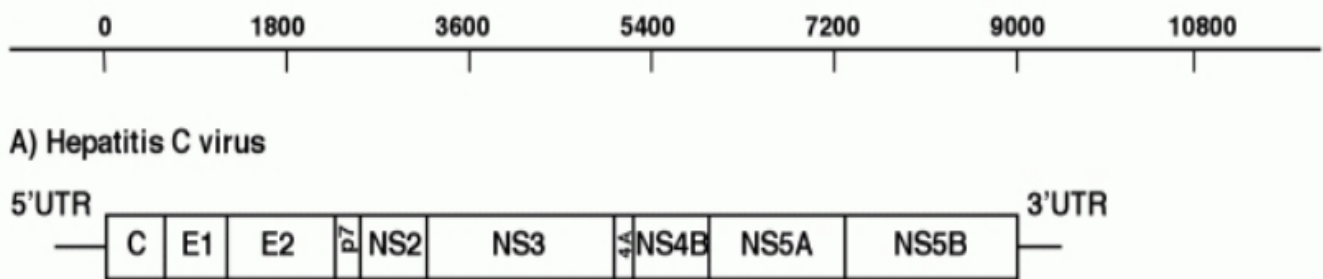

Figure 2: Organization of HCV genome (Courtesy: Miller and Purcell 1990).

The viral nucleocapsid is enclosed by the envelope glycoproteins *i.e.* E1 and E2 which are covalently linked complexes of transmembrane proteins (Hill and Cooke 2014), and the surfaces of these proteins are highly glycosylated (Vieyres et al. 2010). These envelope glycoproteins are necessary for initiation of a disease in a host cell. Additionally, one of the major contents of E1 protein is C-terminal transmembrane domain (TMD) and 4-5 glycosylation sites with 9–11 N-linked sites (El Omari et al. 2014, Vieyres et al. 2010). The E2 protein contains about four O-linked glycosylation sites (El Omari et al. 2014, Falkowska et al. 2007) and 18 conserved cysteines residues (Drummer and Poumbourios 2004). Apart from their glycosylation dissimilarities they both perform as a heterodimer to mediate the viral passage (Falkowska et al. 2007). Furthermore, TMD contains the ectodomain to the virion (Falkowska et al. 2007). The TMD is associated with a perpetuated C-terminal strand towards RBD. The most important cell receptor for all strains of the virus is the binding location for CD81 which is comprised of conserved portions within E2 RBD (Drummer and Poumbourios 2004)**.** There is no HCV immunization however accessible till date, either for prophylactic or therapeutic utility. Improvement of immunizations against HCV still remains a challenge to the scientists. It is one

of the serious reasons for liver cancer and end-stage liver infection, requiring liver transplantation in numerous patients (Perz et al. 2006).

The struggle for the development of new vaccines against hepatitis-C still exists as an extremely troublesome issue since the conventional way of vaccine design that is culture and cultivation of the virus, identification of protective immunogens, preparation of attenuated or inactivated virus or protective immunogen, and determination of molecular composition of the vaccine and its production technology. Further, testing the efficacy of the vaccine on animal model appears impracticable and remains a matter of great concern. No system of HCV replication suitable for virus production in preparative quantities has yet been successful. Constant efforts are being made to prevent transmission of the virus and to develop chemotherapeutic regimens for this leading public health problem. HCV infection is recognized by multiple innate immune pathways, but often not evacuated by immune responses, resulting in a chronic infection and the IFN response pathway is also blocked by HCV through several mechanisms.
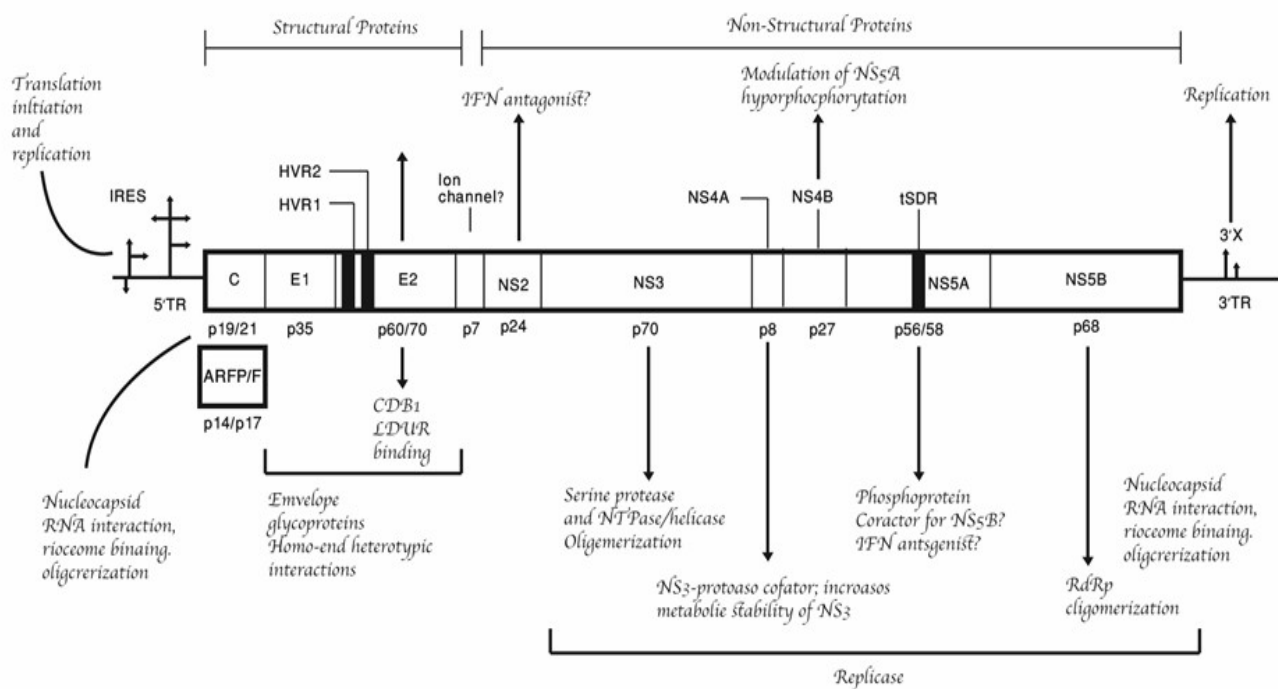
Figure 3. The hepatitis C virus (HCV) genome (Courtesy: Tan et al. 2002)

Evidently, various prospective reports has supported that HCV infection can cause HCC and significantly increases the rate of incidence of the infection among HCV-infected individuals when contrasted with HCV-negative cohorts. The prevalence rate of HCC among HCV-infected persons ranges from 1% to 3% over more than 30 years.

Similarly, HCV infection is linked with a 15 to 20-fold increase in risk for HCC compared with HCV-negative subjects in cross-sectional and case-control studies. It increases the risk for HCC by inducing fibrosis and, eventually into, cirrhosis. Mostly, cases of HCV-related HCC occurs among patient with advanced fibrosis or cirrhosis, making it a dramatic condition listed for HCC surveillance in current recommendations. Once HCV-related cirrhosis is developed, HCC occurs at an annual rate of 1%–4%; although increased rates up to 8% have been reported in Japan Yang and Kim 2015). The incidence of cirrhosis in the later years (20–25 years) after HCV infection ranges from 15% to 35% and is highest among recipients of HCV-contaminated blood products and hemophiliac patients, and lowest among women who received a single dose of contaminated anti-D immunoglobulin. HCC risk also might vary based on the amount of viral load in the contaminated product or repeated exposure. Other risk factors for HCC include the sex of the HCV-infected individual, co-morbidities (co-infection with HBV or HIV, diabetes, obesity, steatosis), viral genotype (HCV 1b), level of alcohol consumption, and age. Among patients with HCV-related cirrhosis, low numbers of platelets or increased levels of -fetoprotein are risk factors for HCC.

3. Viral entry into host

A sequence of events can occur after infection with HCV. The viral entry into the cell is a complex mechanism. The virus attacks hepatic cells via several steps controlled by multiple intracellular signaling events (Duns 2013). It requires four essential receptors and co-receptors *i.e.* CD-81, the scavenger receptor class B type-I (SR-BI), and junction proteins sequences named occludin and claudin-1 (Khan et al. 2014b). The virus particles are also linked with certain lipoproteins such as apolipoprotein E (ApoE), low-density lipoprotein to form the lipoviral particles and high-density lipoprotein (Duns 2013). SR-BI receptor first interacts with ApoE (apolipoprotein E) on the lipoviral molecule of the virus (Khan et al. 2014a), the virus is then mediated into the cell by the action of clathrin-mediated endocytosis process that finally leads to discharge of the viral genome into the host cell (Sharma et al. 2011). E2 is compact and globular in structure, and it does not show any conformation of class II fusion proteins. Earlier it was believed that, E2 is a fusion protein of class II, similar to the other organisms belonging to flaviviruses, for mediating fusion event (Khan et al. 2014a).

4. Infection by HCV

If an individual is found to be infected by HCV then the virus can be detected in the inception of the contamination in serum within 3 weeks, but T-cells or antibodies particular to HCV are recognizable only after an incubation period of 1–2 months (Vernelen et al. 1994), however, this leads to the chronic infection (Park and Rehermann 2014). In that case, chronic infection and re-infection of the virus can be controlled in the acute phase by the role of

neutralizing antibodies (nAbs) (Park and Rehermann 2014), but the delayed response of these nAbs may lead to the chronicity in the patients (Pestka et al. 2007). A HCV vaccine will be successful if it can stimulate both adaptive immune response *i.e.* cellular as well as humoral immune responses. For viral elimination and defense, it has been observed that a protective cellular response is essential (Ashfaq et al. 2011). However, for eliciting a humoral immune response *i.e.* B-cell response against viral infections prophylactic vaccines are preferred, while for activating both humoral and cellular (T-cell) immune responses therapeutic vaccines are favored (Ip et al. 2012).

5. Recent advancement in HCV Vaccine

Peptide vaccines are made of amino acids. A specific antigen is required for the cancer vaccine production along with the immune response boosting factors. This helps in attacking and diminishing the cancer cells. There has been a significant development in the field of peptide vaccines based on the technique of reverse vaccinology. The first peptide-based vaccine clinical trial on melanoma antigen was first carried out in 1995, after that several clinical trials for many other diseases have also been carried out. Previous studies reported that, the peptide vaccines were only limited to the only antigen of human leukocyte. Therapeutic peptide vaccines have several applications due to its site specificity, easy production, safety parameters and it has quiet good consequences in preclinical testing. Recognition of tumor cell antigen stimulates the immune response constantly. Multiple epitope based peptide vaccines can be continuously modified or upgraded to enhance its effectiveness in the treatment of tumor as compared to conventional whole organism based vaccines (Wang and Walfield 2005)s (Wang and Walfield 2005).

Peptide vaccines require a carrier system such as lipid mixtures, bacterial cells, or virus particles for very good drug delivery systems. Peptide vaccines are now extensively and effectively growing with advanced technology and used for the treatment of various ailments like cancer, diabetes, and cardiovascular diseases. For instance, glioma cancer is observed due to the mutation of human epidermal growth factor receptor (EGFR) known as EGFRvIII and is highly expressed. In such cases, chemotherapy results into systemic toxicity and affects adjacent normal cells very drastically. Targeted cancer therapies hold great potential for these problems with enhanced drug potential and efficiency (Gaugler et al. 1994).

In this study the prime objective is to predict peptide vaccine against all genotypes of HCV which will form a part of combination therapy. Peptide vaccines often offer several advantages. To induce strong and protective immunity against viral infections and malignancies, synthetic vaccines need to be administered subcutaneously along with an adjuvant, observed in several murine model systems. Therefore, it is a novel strategy to stimulate immune response very purposefully based on a selected epitope or even a mixture of desired epitopes. Using a suitable adjuvant along

with a peptide vaccine results in very straight forward method of immunization (Hunziker et al. 2001). Recombinant or attenuated viruses always have the high risk of reverse-mutation as compared to the peptide-based vaccines, which do not turn into a dangerous or potential virus as the former does. Peptide vaccines are easily produced at low cost and do not need an uninterrupted cold preservation system unlike traditional vaccines.

Traditional vaccines comprise of either live attenuated or whole inactivated microbes but can be risky and unsafe as virus can turn into active form by reverse mechanism and might produce several side effects, such as inflammation of tissues (Harrison et al. 1999). The vaccine that contains purified or recombinant molecules of the infectious agents like surface proteins or polysaccharides seems to have low side effects and hence less risky in contrast to the traditional ones (Chakraborty 2014). In case of pathogens where no vaccine is either yet available for example against HCV or difficult to develop on the basis of the pathogen's polysaccharide, the first attempt was made to develop a peptide-based multiple-epitope loaded vaccine as a preventive measure against the pathogen for immediate application using the modern tools of proteomics. Vaccine designing, either peptide or DNA vaccine, using bioinformatic tools is a risk-free and rapid process to design the effective vaccines against many deadly diseases. Since, peptides have shorter half-life *in-vivo*, novel drug delivery system has been developed with alteration in peptide sequences. To overcome the obstacle peptides are being utilized by the means of bearers like radio-nuclide carriers, cytotoxic drug or by targeting drug to tumor directly.

Other advantages of peptide-based vaccines include:

(a)   T cell responses induced by the vaccine can be directly screened and supervised

(b)   epitope introduction may avoid antigens that may have non-therapeutic autoimmune activity,

(c)   directly triggers strong CD8+ T cell response,

(d)   specific screening of the patient's immune reactions, and

(e)   regular booster dose application of vaccines.

A peptide-based vaccine is often associated with some demerits such as:

(a)   Class I MHC restriction that limits the effectiveness of particular peptide to certain HLA types, sometimes short peptides may bind to MHC on non-professional APCs directly (which may induce tolerance),

(b)   degradation of peptide in absence of an adjuvant. Adjuvant is often needed to protect the peptide from protease degradation and direct it into an immune response pathway, although immune responses may be of low magnitude.

In the late 20th century, most of the known vaccines developed had their roots in traditional process, but this process had several limitations. Scientists searched for new methods to develop vaccines against disease causing

pathogens. Exploring beyond the principles given by Pasteur, they used computers to analyze genomic data of pathogens to design desirable vaccines and forwarding them for further clinical trials without the need of growing them in laboratories. A break-through revolution came with the introduction of new technologies like recombinant DNA technology for vaccine production. In 1995 Craig Venter first made public the genome of the first free-living life form *(Fleischmann et al. 1995).* Advances in use of conjugated polysaccharides, adjuvants and capacity to access the pathogen's genome paved the way for development of a novel technology to harness genomic data to design peptide vaccine and this new technology was coined as "reverse vaccinology" (Fleischmann et al. 1995, Rappuoli 2000).

6. Epidemiology of HCV

Amino acids are the main building blocks of peptide vaccines. The peptide components of vaccine contains about 10-30 amino acids including the specific epitope of an antigen found in an infectious agent. Studies showed that peptide vaccine can target viral diseases and even some allergies (Nava-Parada et al. 2007). There are several vaccines discovered till date after Edward Jenner pioneered the concept of vaccine and discovered it against small pox in late 1760s (Ingolotti et al. 2010). A few decades later, Louis Pasteur started working on vaccines after the discovery of the fact that many diseases are caused by microbes, and developed the basic rules of vaccinology (Yang and Kim 2015).

The idea of triggering the immune response of a patient against any type of cancer has been proposed long back. William Coley was the pioneer for initiating work on immunotherapy for proposing a basic treatment of cancer during 1890 to 1891. Coley first observed the tumor development, and later infused a strain of live *Streptococcus pyogenes* into the lump of the patients. He then hypothesized the fact that the body would battle against the contamination and demolish the tumor by the method of "collateral damage". After the administration of the regimen the patients developed severe fever and headache in conjunction with bacterial sepsis. As per the observation, Coley suggested that, the tumor showed a decreasing pattern in the size due to hemorrhagic necrosis. But the test brought about a number of deaths due to bacterial sepsis. Later, Coley altered the investigation on immunization by utilizing the filtrates and made bacterial cells free, that too in a combined culture of *Streptococcus* and *Serratia marcescens* (Coley 1891). Later, Telaprevir (TVR), Direct-acting antivirals (DAAs), and Boceprevir (BOC) were the first ones to be licensed against HCV genotype 1 infection in 2011. The effectiveness of the triple therapy regimens proved to be useful to some extent with certain side effects as well such as fever, nausea with additional psychiatric effects of insomnia, fatigue, headaches, irritability, and depression were reported. Because of the high genetic variability, resistance to DAA started increasing and cropped up as a new problem. Significant drawbacks of the recent therapies (including the DAAs) are after-effects, high cost, and delayed identification of the ailment (which

commonly appears after a long time of persistent infection). Numerous patients initially develop irreversible liver problems that finally lead to liver cancer. It has been estimated that with an aging population by 2030, the frequency of cirrhosis and cancer is likely to be tripled in the developed nations.

Vaccines against diphtheria and tetanus were developed after Ramon and Glenny in the year 1923. They first isolated the required components from the cultures, inactivated them, and directed for the development of DTP & DTaP subunit-based vaccines composed of purified antigens from *Corynebacterium diptheriae, Clostridium tetani* and *Bordetella pertussis.* It was in the year 1948, when DTP was licensed by the FDA in United States. After that, DTP vaccine became the first version of a collective diphtheria, tetanus and pertussis bacterial vaccine that was routinely administered to children from the 1940's to the mid 1990s (Fine 2003).

In 1982, the first synthetic vaccine for Diphtheria was developed from diphtheria toxin by Louis Chedid. The production of diphtheria vaccine involved synthesis of three peptides corresponding to the fragment of diphtheria toxin. They included already reported several structural analogs, tetradecapeptide, hexadecapeptide and octadecapeptide. Peptides or synthetic carriers were introduced in guinea pig for protection against the vigorous activity of diphtheria toxin. The conjugation of peptides showed a positive immune response toward the octadecapeptide and was observed in mice (Maione et al. 2005).

The first synthetic vaccine developed against malaria was SPf66 produced by Manuel Elkin Patarroyo in 1986. The SPf66 synthetic polypeptide vaccine was based on *Plasmodium falciparum. Studies* showed that immune response to SPf66 was not dependent of age and the efficacy of vaccine varied depending on person-time of exposure (Lamabadusuriya 2009).

Moreover, for the rapid vaccine development, reverse vaccinology method is widely applied against several infectious agents. With the advent of this procedure, it is now possible to predict epitopes of high efficacy without the need for culturing any extremely infectious agent. It also made possible, the studies on antigen structure and function without direct contact with the extremely infectious agent. The prime requirement of this technique is the availability of whole genome sequence of that infectious organism.

In 2009, during the H1N1 outbreak, the company Novartis Vaccine and Diagnostics first put forward the synthetic approach of vaccine designing. Furthermore, monovalent vaccine for influenza A (H1N1) was licensed for the first time by the FDAs (Control and Prevention 2009b). The company manufactured the vaccine in the similar way, as it was used for the manufacturing of its seasonal trivalent inactivated vaccine. They first experimented with a two-dose regimen of 15 μg-30 μg antigen of hemagglutinin, because of the uncertainty about the fact that whether a high antigen preparation

or a two-dose series will produce a satisfactory immune response (Clark et al. 2009). The vaccine either contained live attenuated monovalent vaccine (LAMV) for nasal induction or monovalent inactivated split-virus or subunit vaccines for injection (MIV) (Control and Prevention 2009a).

Using reverse vaccinology approach, the first pathogen against which the synthetic vaccine was prepared was for serogroup B *Neisseria meningitides (Kaboré et al. 2012).* Until now, only single candidate vaccine for HCV was aimed at eliciting neutralizing antibodies (nAbs) and was tested in human volunteers. In a virus, the envelope may have more chances for neutralizing antibody triggering vaccine, while early studies suggested that the epitopes of the envelope protein are highly variable amongst infected individuals (Sarobe et al. 2003, Ray et al. 2010)).Chiron Corporation pioneered the vaccine based on recombinant DNA technology. Glycoproteins E1 and E2 were extracted from cells of mammals as a weapon. Akazawa has successfully demonstrated that sufficient cell culture derived HCV (HCVcc) can be produced (Akazawa et al. 2013 [1]). Akazawa first inactivated the HCV and then immunized mice. The antibodies released in the body of mice was observed to neutralize the HCV genotypes that is 1a, 1b, and 2a viruses that showed positive results and prevention against the infection in human liver transplantation on the uPA$^{+/+}$ SCID mice model but only at low doses of the infection. He then collected the immunized serum from the body of model mice and immunized it with the attenuated HCVcc substance and found it more effective and reliable at neutralizing the homologous viral challenge as compared to the immunized serum collected from the mice model vaccinated with the recombinant-E2 protein only or may be with both recombinant-E1/E2 simultaneously**.** Later, the part of antibodies in defense against infection by HCV was studied by the mechanism of passive immunization in both animal and human models. This was observed in individuals with commercial intravenous immunoglobulin (IVIG) applications administered to elicit primary immune response. The exclusion of plasma containing anti-HCV made it irresistible than former clusters of IVIG produced from unscreened plasma and so it started losing its effectiveness. Clinical trials based on this technique are being continuously tested for observing the adequacy of HCV nAbs to avoid re-infection in liver transplant patients.

In the portfolio of vaccines, we cannot forget about polio vaccine that not left so far behind. This vaccine sets an example for a combination vaccine that provides protection against multiple variants of a single disease. Hence, the inactivated polio vaccine (IPV) is developed by the combination of inactivated poliovirus types 1, 2, and 3 vaccine strains (Thompson and Duintjer Tebbens 2014).

7. Conclusion

HCV-induced hepatocellular cancer causes significant morbidity among affected individuals worldwide. Quite a good number of studies were done on HCV epitopes of different genotypes. The studies revealed the requirement of using immunoinformatics as a good predictor for identifying both T cell and B cell epitopes, along with their immunogenicity potential and other biochemical properties of epitopes. Reverse vaccinology opened up a new dimension in the field of vaccine prediction using bioinformatics. The basic idea behind this technique is to use the complete genomic sequence of an organism to predict potential antigens for a candidate vaccine with the help of prediction algorithms. For any synthetic vaccine, immune dominant epitopes must be present for triggering immune response against the pathogen. With the advent of reverse vaccinology, it is now possible to predict epitopes of high efficacy without the need for culturing any extremely infectious agent. It also made possible the studies on antigen structure and function. The prime requirement of this technique is the availability of whole genome sequence of an infectious organism. However, known facts are not conclusive enough for understanding the etiology the infection. More research and epidemiologic data are required for in-depth knowledge of the diagnostic criteria and its improvement, the natural course of the disorder, as well as to validate any strength of true associations with comorbid syndrome or disorder, as the existing data is not conclusive enough to determine the accurate prevalence.

Abbreviations

aa	Amino acid
ANN	Artificial Neural Network
APC	Antigen Presenting Cell
ApoE	Apolipoprotein E
BOC	Boceprevir
CombLib	Combinatorial Peptide Libraries
CTL	Cytotoxic T Lymphocyte
DAAs	Direct Acting Antiviral Agents
EGFR	Epidermal Growth Factor Receptor
GMP	Good Manufacturing Practices
HCC	Hepatocellular Cancer
HCVcc	Hepatitis-C virus Cell Culture
HCV	Hepatitis-C virus
HIV	Human Immuno deficiency Virus

HLA	Human Leukocyte Antigen
IEDB	Immune Epitope Database
IFN	Interferon
pl	Isoelectric Point
IVIG	Intravenous Immunoglobulin
sLC_MS	Mass Spectrometry
MHC	Major Histocompatibility Complex
Mw	Molecular weight
nAbs	Neutralizing antibodies
NCBI	National Centre of Biotechnology
NASH	Non-alcoholic Stearohpatitis
NS	Non-structural
Nt	Nucleotide
ORF	Open Reading Frame
RBD	Receptor Binding Domain
RNA	Ribonucleic acid
SL	Stem-Loop structures
SMM	Stabilized Matrix Method
Sr	Scavenger Receptor
SVR	Sustained Viral Response
TVR	Telaprevir
TMD	Terminal Transmembrane Domain
UTR	Untranslated region
WHO	World Health Organization

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