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Hepatitis C - Challenges and New Approaches in Vaccine Development

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Abstract

Hepatitis C continues to be the serious global health treat and hundreds of people die every year due to liver failure. Hepatitis C Virus, the virus that that won the battle with no vaccine till date to beat its survival. Despite 15 years of its discovery, no vaccine has come up eradicate the virus. The high genetic diversity and the resistant nature of the virus is halting the progress towards vaccine development however many promising new antiviral drugs are in clinical trials giving a hope that in the coming years a successful vaccine will be developed. Many approaches like inducing neutralising antibody, inducing T cell response, development of Direct Acting Antivirals and drugs targeting host proteins are being tried out for the development of a safe and effective vaccine for Hepatitis C, some of which are discussed in this review.

Keywords: Hepatitis C, Vaccine, Antibodies, T cell response.

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1. Introduction

Hepatitis is a condition defined as the inflammation in the liver. Hepatitis is most commonly caused by viral infection with the individualisation of the different types of hepatitis emerging after World War II. There are 5 main hepatitis viruses referred to as types – A, B, C, D and E. Dr. Blumberg and his colleges identified the Hepatitis B virus in 1967 and two years later a vaccine was invented by the same group. In 1976, Dr. Blumberg won the Nobel Prize in medicine for his discovery. Currently vaccines are available only for hepatitis A and Hepatitis B.

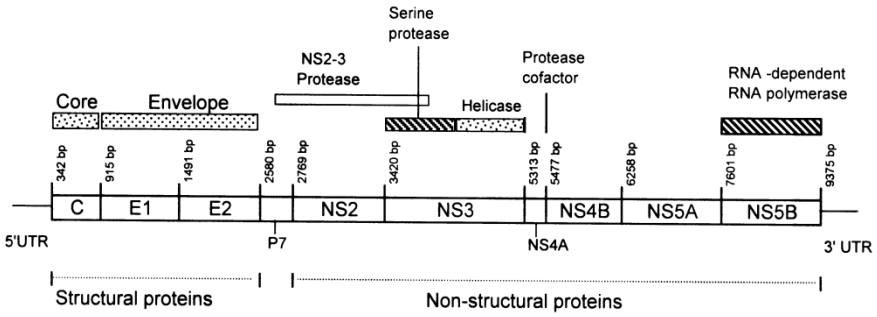
Hepatitis C Virus (HCV) has been identified as the major cause for the non-A and non-B hepatitis in 1989 and it is the leading cause of the chronic liver disease in industrialised and developing world [1]. Hepatitis C is a major concern and medical burden globally, infecting more than 200 million people worldwide. A global prevalence of infection is estimated at 2% to 3% [2-4]. Approximately half of the global HCV-infected subjects confined to China, India, Egypt, Pakistan and Indonesia. The high prevalence of HCV infection demands an urgent need for the development of new treatment modalities. The common modes of spread of infection are blood transfusions, injection drug use, unsafe therapeutic injections and healthcare-related procedures. The acute illness is generally mild and is not clinically recognisable with the initial non-specific flu like symptoms which are not diagnostic to HCV as they are common to many viral infections. The infection is defined as chronic after six months of the persistence of the viral RNA in the blood stream. This transition from acute to chronic hepatitis C is usually sub-clinical.

2. HCV genome organisation

HCV is an uncapped positive single standard RNA virus belonging to flaviviridae family. The genome is approximately 9.6kbpairs with an uninterrupted open reading frame (ORF) encoding a single polyprotein of 3000 amino acids (Figure 1). The polyprotein is further cleaved by host and viral proteases to form the 10 viral proteins that constitute the structural and nonstructural (NS) components of the virus (listed in Table 1) [5]. Core, envelop glycoprotein E1 and E2 constitute the structural proteins;

the viroporin p7 required for virus particle formation [6]; NS2 protein mediates cleavage between NS2 and NS3 and is also required for virion assembly; NS3 protein, composed of protease and helicase domains that are required for polyprotein processing and RNA replication respectively; NS4A protein acts as a cofactor of NS3 and activates NS3 protease activity; NS4B that induces membrane alterations; the multifunctional NS5A protein is required for replication and assembly; and the NS5B protein that has the RNA Dependent RNA polymerase activity. The structural proteins as well as p7 and NS2 are processed by host cell signal peptidase and signal peptide peptidase [5], whereas the remainder of the polyprotein is processed primarily by the NS3 protease. Apart from the ORF, the viral genome has 5' and 3' untranslated regions (UTRs) that contain secondary RNA structures essential for viral replication. The 5' UTR has an internal ribosome entry site (IRES) that initiates the translation.

Figure 1: **HCV genome Organisation -**



Schematic of HCV genome organization showing the location of HCV genes and proposed functions of gene products. 5' and 3' non-coding regions (NCR) are indicated as shown. Numbering refers to nucleotide positions of genes, based on the sequence of HCV genotype.

HCV replication occurs in cytoplasm in a membranous compartment called 'membranous web' formed by the viral proteins NS3, NS4A, NS4B, NS5A, and NS5B. The NS5B polymerase carries out the error prone replication of the RNA genome. The newly assembled virions associate with Very Low Density Lipoprotein or Low Density Lipoprotein components, acquire the envelope by budding from the Endoplasmic Reticulum and are secreted as lipoviroparticles [7-10]. These released particles are now ready to infect new host cells to spread the infection.

Table 1: The Viral proteins and their respective functions are listed along with the different classes of drugs.

Viral Factor	Virological Function	Drug Classes
E1/E2	Envelope glycoprotein, cell attachment and cell entry	Neutralizing antibodies
p7	Viroporin; involved in assembly and release of infectious virions	Amantadine- imino sugars
NS3/4A	Serine protease; processing of viral polyprotein; interference with innate immunity	noncovalently binding linear inhibitors; macrocyclic inhibitors
NS4B	Membrane remodelling	Various small-molecule inhibitors; silibinin
NS5A	RNA replication, assembly of virus particles, induction of double membrane vesicles	NS5A-inhibitors
NS5B	RNA-dependent RNA-polymerase	Nucleosidic inhibitors (NI); Nonnucleosidic inhibitors (NNI)

3. Genotype distribution

As of now there are 7 major HCV genotypes and 120 subtypes of the virus [11]. The genotypes 1-3 has worldwide distribution .The subtype 1a is common in United States and Europe and subtype 1b is predominant in Japan [12]. The subtypes 2a and 2b are common in North America, Europe and Japan, whereas subtype 2c is common in northern Italy [12]. HCV subtype 3a is the most common subtype in India [13], Pakistan [14]and Nepal [15]. Genotype 4 is seen in Central Africa and Middle East. To the best of our knowledge the genotype 5a is reported only in India [16] and Brazil [17]. Genotype 6 in South East Asia [18] and only one case of genotype 7 is reported in Canada from a central African immigrant [19].

4. Treatment regimens for HCV

As of now there is no effective vaccine for HCV infection with the current standard of care being the use of pegylated interferon and ribavirin. The interferon alpha therapy has side effects like thyroid dysfunction by producing anti thyroid autoantibody and ribavirin cause haemolytic anemia [20, 21]. In 2011, two protease

inhibitors Teleprevir and Boceprevir obtained regulatory approval and marked the beginning of the clinical use of HCV-specific direct acting antiviral (DAA) [22]. These 2 protease inhibitor are effective and well tolerated to genotype 1 with the major limiting adverse effect of anemia [23]. The two treatments discussed above often leads to early termination of the treatment resulting in suboptimal treatment. One reason being the diversified genetic nature of the virus leading to quasispecies formation due to the error prone replication of the virus. It creates substitutions in viral genome that confers resistance or protection to the virus leading to treatment failure. Other is that the new therapies are cost effective and treatment of the large number of infected individuals imposes a major burden to the health care system. This is a major limitation in the developing countries and even the developed countries cannot afford to treat all infected patients. Therefore there is a prior requisite for the development of vaccine to prevent HCV infection.

Despite of drawback of genetic diversity of the virus, several antiviral products are in clinical trials and the treatment regimen mainly include the use of direct acting antiviral, developing neutralizing antibodies, inducing the T cell response, drugs targeting host proteins. Many shades of gray still remain in understanding the HCV biology and in the direction of the treatment.

4.1 Direct Acting Antivirals

The direct acting antivirals came into action when FDA approved the two NS3/4A protease inhibitors named telaprevir and boceprevir for treatment in combination with PEG-INF/RBV. Boceprevir, Telaprevir and Simeprevir (TMC435) are the first generation antivirals approved by FDA and many other direct acting antivirals are in clinical trial. The second generation protease inhibitors MK-5172 and ACH-2684 are in phase II clinical trial. Sofosbuvir is one of the nucleotide inhibitor approved as a “interferon-free” drug by FDA in United States [24]. This oral drug is priced by Gilead as $1000 per pill in the US, this high cost of the drug will make the treatment of Hepatitis C unaffordable in many developing countries.

The DAA have several disadvantages example the phase III drug Asunaprevir which is a NS3 protease inhibitor shows symptoms like Diarrhoea, nasopharyngitis and headache and discontinuation in occurred in two patients due to hyperbilirubinemia and transaminase elevations [25]. The other limitation is that a single-nucleotide substitution is sufficient for HCV resistant to these drugs [26, 27]. The other drugs in clinical trial include the nonnucleotide inhibitors ABT-333, BI207127 and the NS5A inhibitors GS-5885 and ABT267. These drugs are cost effective [28] and their side-effects lead to early termination of the treatment demanding a need for the safer and effective vaccine. DAAs are not effective if HCV is diagnosed at a late stage and in cases of hepatocellular carcinoma. The safety and efficacy of DAAs in children and pregnant women is yet to be determined [29]. These disadvantages pave the way for exploring candidates targeting the other HCV viral proteins.

4.2 Prophylactic and Therapeutic Vaccines

Different preclinical studies have been carried out in the development of prophylactic and therapeutic vaccines within the last two decades using different strategies and targeting different regions of the HCV polyprotein. The list of different candidates in clinical trial is given in Table 2. One strategy uses the improvement of T cell responses as it is known that the early collapse of the CD4+ T cell response impairs the antibody production and CD8+ T cells responses lead to viral persistence [30]. Thus an effective vaccine should be able to induce long-lived CD4+ T cells response to fight the virus back.

Studies by Folgori *et al* showed that immunisation by MRKAd6, coding for HCV NS proteins, followed by boost immunisations of MRKAd24NSmut and later a plasmid DNA encoding NS proteins in chimpanzees showed vigorous T cell responses and immunized animals cleared the virus after infection [31]. Based on this a phase I clinical trial with 40 healthy individuals was completed to address the safety and efficacy. Following this, a phase I and phase I/II clinical trial is currently recruiting participants (NCT01296451, NCT01436357). A recent study has also showed that immunization of mice with the DNA vaccine encoding NS regions (NS4A, NS5A and NS5B) showed induced HCV specific CD4+ and CD8+ T cell responses, upregulation of IFN gamma and clearance of the HCV virus from the hepatocytes [32]. However a comparative analysis between the DNA and peptide approaches showed that immunization with peptides of nonstructural regions showed efficient viral clearance, long lasting immune response and increased antibody in comparison with DNA based immunisation [33]. A first-in-man study by Barnes et al showed HCV-specific T cells induced by ChAd3 are optimally boosted with MVA (Modified Vaccinia Ankara), and generate very high levels of both CD8(+) and CD4(+) HCV-specific T cells targeting multiple HCV antigens[34]. In the study they have used a heterologous prime-boost vaccination strategy based on a replicative defective simian adenoviral vector (ChAd3) and MVA vector encoding the NS3, NS4, NS5A, and NS5B proteins of HCV genotype 1b[34].

The other strategy actively being worked on is employing recombinant proteins targeting HCV polyprotein. This is based on the studies from chimpanzees in which chimpanzees immunised with the envelop glycoprotein E1E2 generated a strong immune response

that protected against HCV genotype 1a [35-37]. The results of the phase I clinical trial (NCT00500747) initiated after this study addressing the safety and efficacy of the vaccine in healthy humans showed that the vaccine induce strong humoral and CD4+ T cell response [37].

The prophylactic vaccine targeting envelop proteins induce neutralizing antibody (nAb) response. These nAb are the main components of the host defence during viral infections and they confer protective immune response against infection. The antibodies generated during acute infection target structural and nonstructural proteins. Several studies have shown a strong correlation between viral clearance and development of nAbs response in the acute phase of the disease [38, 39]. Majority of nAb have been mapped for the enveloped region [40-44]. Studies have shown that Monoclonal antibody against E1E2 regions prevents viral entry in cell culture [45, 46]. The protective effect of nAb gives a hope for use as a therapeutic option. However, a combination of T cell based vaccines and glycoprotein based vaccines can be tested to boost the efficacy and to fight against the Virus.

Table 2: List of different approaches and the candidates in clinical trial

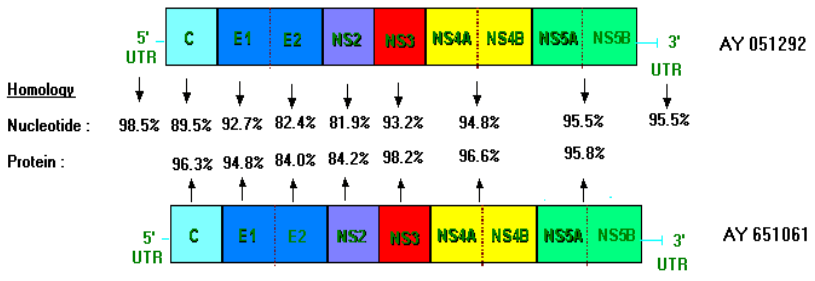
Approach	Prophylactic/ Therapeutic	Immunogen-Adjuent	Stage of Development	Current Status	Reference
Peptides/ proteins	Prophylactic	E1E2	Chimpanzees	Published	[35]
					[36]
		Core-iscomatrix™	Phase I(30 volunteers)	Published	[47]
		E1E2-MF59C.1	Phase I(60 volunteers)	Published	[37]
		E1E2	Phase I(50 volunteers)	Recruiting	[39]
		Core, E2, NS3, NS5B	BALB/C mice	Published	[33]
DNA vector	Prophylactic	Ad6NSmut DNA-Nsmut	Chimpanzees	Published	[31]
		Ad6NSmut ChAd3NSmut	Phase I (40 volunteers)	Published	[48]
Viral vector	Prophylactic	AdCh3NSmut MVA-Nsmut	Phase I/II(68 + 276 IDU)	Recruiting	[34]
	Therapeutic	AdCh3NSmut MVA-Nsmut	Phase I(19 volunteers /14 patients)	Recruiting	[34]
		Ad6NSmut ChAd3NSmut	Phase I (32 patients)	Completed	[39]
		Ad6NSmut MVA-Nsmut + SOC	Phase I (9 patients)	Completed	[39]

5. The Indian aspect

To the best of our Knowledge limited studies with respect to vaccine development in India and the vaccine initiatives worldwide are focused on inducing nAb production against E1, E2 proteins which are effective only for HCV genotypes 1 and 2. These vaccine is the purified envelope proteins of genotype 1a strain and elicits antibodies that can cross-neutralize the *in-vitro* infectivity of heterologous strains derived from genotypes 1a, 1b, and 2a [37, 38]. However, the genotypes most prevalent in India are genotypes 1 and 3. South India shows high prevalence of genotype 1 followed by 3 whereas in north India genotype 3 is prevalent followed by genotype 1. Researches focused on development of vaccine for genotype 3 are limited.

Our group have completely sequenced two HCV genomes isolated from two different patients and submitted to the GenBank (Accession numbers: AY051292 and AY651061). Pairwise alignment of the Indian strains AY051292 with AY651061 showed more than 90% similarity at the protein level in the nonstructural region of the virus. (Figure 2)

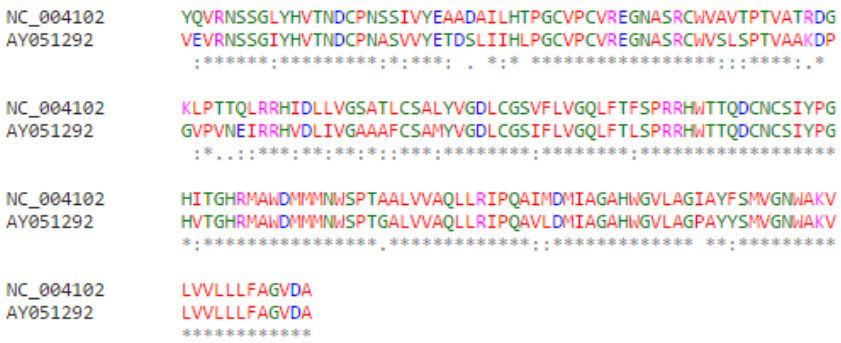
Figure 2- Pair wise comparison of the two Indian HCV sequences



Homology at the nucleotide and protein levels is shown as percentage. UTR stands for untranslatable region.

Comparison of the complete amino acid sequence of the AY051292.1 (India) with NC_004102.2(USA) showed 85.38 % identity. Analysis of E1 and E2 regions of these 2 strains showed 80.73% and 79.06% identity respectively (shown in Figure 3 and 4 respectively). Comparision of the NS5B region of AY051292 with the NC_004102.1 showed 86.10 % identity (shown in Figure 5).

Figure 3: Sequence alignment of the E1 protein



The figure depicts Comparision of Amino acid Sequence E1 protien of AY051292.1 and NC_004102.2, where * indicate same aminoacid in the two sequences.

Figure 4 - Sequence alignment of the E2 protein

The figure depicts Comparision of Amino acid Sequence E2 protien of AY051292.1 and NC_004102.2, where * indicate same aminoacid in the two sequences.

Figure 5: Sequence alignment of the NS5B protein

The figure depicts Comparision of Amino acid Sequence NS5B protien of AY051292.1 and NC_004102.2, where * indicate same aminoacid in the two sequences.

Amino acid Sequence alignment by MUSCLE software of the complete genome of HCV 1a from USA (accession number NC_004102.1) with the Indian complete genome HCV 1a (accession number AY051292) resulted in percentage identity of 85.39%.

As it is well known that the vaccines developed by the western world are exorbitant, so it will be beneficial outcome if a vaccine is for HCV is developed in India as it is economical and affordable by the developing nations.

6. Conclusion

Even two decades after the discovery of virus, a safe and effective vaccine for Hepatitis C virus is not yet developed. Most of the studies done by the western world are focused on the genotypes which are most prevalent in their respective countries. The drugs discovered by the western countries are expensive and citizens from developing countries like India could not afford to buy. Research on Indian genotypes is limited and focused on clinical applications and disease monitoring. Apart from this, a recent cross-sectional study by Dr. Sunil Suhas Solomon shows a high burden of HCV infection, coupled with a lack of awareness and poor access to care among people who inject drugs in India [49]. Research in terms of developing an effective and affordable vaccine candidate for treatment should be a priority and of prime focus in India. Besides research, an increase in awareness and broad screening programmes in India would definitely help in providing quality care to patients. Although India is not yet there in developing a vaccine for HCV identifying more candidates that can elicit nAb and candidates that can increase the T cell responses will pave way for the development of a cheap and effective vaccine in India.

Conflict of interest

The author declares no conflict of interest.

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