

## Different expression systems for production of HCV structural proteins

Masoumeh Mirnurollahi<sup>1</sup>, Shiva Irani<sup>1</sup>, Azam Bolhassani<sup>2,\*</sup>

<sup>1</sup> Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

<sup>2</sup> Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran

\*Corresponding author: email address: [A\\_bolhasani@pasteur.ac.ir](mailto:A_bolhasani@pasteur.ac.ir) (A. Bolhasani)

### ABSTRACT

Hepatitis C virus (HCV) is an important agent causing chronic liver infection, which often leads to liver cirrhosis and lethal hepatocellular carcinoma (HCC). At present, there is no effective HCV vaccine for prevention of hepatic disease and the standard treatment is neither economical nor fully effective in all the patients. However, vaccination based on structural and nonstructural proteins of HCV has attracted a special interest. Different heterologous systems have been used to generate the recombinant HCV core, E1, and E2 proteins including *Escherichia coli*, yeast, insects and mammalian cells. Further studies showed that the amounts of HCV recombinant proteins in *E. coli* are more suitable and un-expensive compared to other systems. It should be considered that this system is not efficient for generation of the glycosylated proteins. Thus, the structure of proteins is an important agent of selection for expression systems. The selection of expression systems will be critical for the use of recombinant proteins as an immunogen. In this mini-review, we briefly describe different expression systems for generation of the HCV recombinant structural proteins applied in vaccine design.

**Keywords:** HCV; Expression systems; Core e1e2

### INTRODUCTION

Hepatitis C virus (HCV) infection is a major medical problem both in developed and developing countries [1, 4]. HCV is the member of the Flaviviridae family and the viral products (Core, E1, E2, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) are produced from a 3000 amino acid polyprotein expressed from a single open reading frame [1, 2, 4]. This polyprotein cleaves to structural and non-structural proteins using cellular and viral protease. The HCV structural proteins include the nucleocapsid or core protein (29) and the two envelope glycoproteins, E1 and E2 [3]. The HCV core protein regulates the cellular processes, contributes to viral replication and pathogenesis and expresses membrane protein features [5]. A tendency to aggregation is a characteristic of the HCV glycoproteins, and it may play a key role in virus pathogenesis [3]. E1E2 heterodimers also can bind the virus to the cell and then enter it [3]. These proteins can release neutralizing antibodies against HCV infections in the host and thus, core, E1 and E2

proteins are suggested as the main vaccine candidates [4]. The non-structural proteins are thought to generate the viral replicase complex [5]. The C-terminal component of the polyprotein contains non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) that provide functions necessary for synthesis of viral RNA and also assembly of infectious particles [6-8]. Recently, improvement of vaccine strategies against HCV infections has attracted a special interest. Protein vaccination is a major approach for prevention of HCV infections [29,30]. One of the most important subjects in design of protein vaccine is the selection of suitable expression systems. Herein, we describe some different expression systems for generation of HCV structural proteins.

#### **HCV vaccines**

Up to now, there is not any vaccine for prevention of HCV infections, because of the high levels of strain variations and low efficiency of virus replication in appropriate cell cultures [3]. However, development of an effective vaccine

has likely critical role in the control of HCV infections [2]. There are two main approaches for HCV vaccine development, including prophylactic and therapeutic vaccines for clinical use [9]. Structural proteins were known as the main vaccine candidates [1]. Indeed, the recombinant HCV core protein could be presented as a major vaccine candidate [5]. Generally, HCV vaccines are further designed as DNA-, peptide- and recombinant proteins which are currently underway in phase I/II human clinical trials [9]. Currently, the primary step to develop an HCV vaccine was conducted toward the production of a recombinant protein as a subunit vaccine [9]. Thus, the use of different expression systems and the comparison of their efficiency are a special interest for researchers. Different heterologous systems have been applied to produce the recombinant HCV core and E1, E2 proteins such as *Escherichia coli*, yeast, insect cells, and mammalian cells [5] which are discussed as follows. The studies showed that some recombinant proteins are enough to induce immune response but others require adjuvant therapy [8]. For example, the combination of core protein with hsp90 as an adjuvant elicited high levels of total IgG and IgG2a immune responses [10, 12]. Also, the studies indicated that core+1 formulation with various adjuvants may induce different profiles of immune responses (Th1 or Th2) [11].

#### ***Escherichia coli***

Different *E.coli* strains such as BL21 (DE3), K12, M15 and transcriptional control systems were being used to produce high levels of the proteins [13]. The recombinant HCV core proteins were efficiently expressed in *E.coli* as various fusion forms carrying the His-tag, the maltose binding protein (MBP) and glutathione S-transferase (GST). Generally, bacterial expression systems offer higher yield at significantly lower cost compared to other expression systems [14, 27, 28]. The authors showed that during viral morphogenesis, HCV core protein is cleaved by cellular signal peptidase into the truncated core proteins of 191 and 173 with molecular weights of 21 and 19 kDa, respectively [20, 21].

It has been shown that *E.coli* BL21 (DE3) plyS strains were used for production of the recombinant core protein using IPTG inducer.

This system was able to generate  $\sim 25 \pm 1$  mg/l of the purified core protein. However, the formation of inclusion bodies (IB) has been considered as obstacles for development of the mature HCV core protein in *E.coli*. Therefore, many investigators have developed the truncated core protein in *E. coli* or its expression in other hosts [15]. In other studies, *E. coli* BL21 was applied as the recombinant expression system that was induced by arabinose. This system was able to achieve yields around 2.5 or 3.5 mg/l of the purified core protein (HCV core 173) [13, 16]. The typical yields of purified core protein from a one liter *E. coli* BL21 (DE3) bacterial culture using IPTG inducer were 10 and 5 mg for HCV core 124 and HCV core 179, respectively [17]. In addition, the expression of core protein as linked to GST using *E. coli* BL21 (DE3) cells was directed by adding IPTG. This simple procedure for purification of the GST-core (1-123) fusion protein usually resulted in yields of 50-100 mg protein/liter of culture medium [14]. Recombinant core proteins (r-core) were able to detect anti-core antibodies in HCV positive serum samples. HCV core was able to elicit a broad range of antibody depending on the immunogen conformation [13, 12]. In other report, the rate of expressed E1, E2, and E1E2 proteins using IPTG inducer were about 30, 50, and 60%, respectively. Both purified E1E2 chimeric proteins and E2 protein could interact with a putative cellular receptor CD81, while purified E1 protein could not interact with CD81. The sera of rabbit immunized with the E1E2 inhibited the binding of E2 protein to the major extracellular loop of human CD81 and reacted with both E1 and E2 proteins, respectively. Anti-E1 and E2 antibodies could be generated simultaneously in the rabbit immunized with the E1E2, and the titers of antibodies were 63 or 56% higher than the titers induced by E1 or E2, respectively [22]. Our group also showed that the BL21 strain was more potent than Rosetta strain for generation of HCV core protein in pET28a expression system. On the other hand, M15 strain was suitable for expression of coreE1E2 in pQE-30 bacterial system.

Herein, the purification of HCV core and core-E1E2 proteins was performed using the reverse staining procedure without the need of any chromatography columns [29].

### **Insect cells**

The *baculovirus*-insect cell expression system has two features which make it suitable for HCV protein expression: a) the eukaryotic insect cells possess several co- or post-translational modifications, including fatty acid acetylation and glycosylation, similar to mammalian cells; b) unlike many mammalian cell expression systems, the *baculovirus* expression system produces high levels of heterologous proteins [3]. In order to expression of HCV structural proteins, Sf9 insect cells (*Spodoptera frugiperda*) were co-transfected with wild type *baculovirus* [23]. Furthermore, the production of HCV-like particles in insect cells was performed using a recombinant *baculovirus* containing the cDNA of the HCV core E1E2 structural proteins [24].

### **Yeasts**

*Pichia pastoris* strain as an efficient yeast system was used for HCV protein expression. It has been reported that HCV proteins generated in *Pichia pastoris* induce strong immune responses in animal models.

This system is also faster, easier, and less expensive than expression systems derived from higher eukaryotes including insects and mammalian cells and generally produces higher levels of protein expression. But some authors reported that it was difficult to express HCV envelope proteins by *Pichia pastoris* [1]. In one study, low levels of protein expression (*e.g.*, HCcAg.120 or HCcAg.176) were observed in the recombinant *Pichia pastoris* cells [25]. In addition, *Pichia pastoris* strain GS115 was used for expression of core E1E2 protein by methanol inducer [1]. In other study, *Pichia pastoris* strain SMD1168 was used for expression of E1E2

proteins under induction by methanol. The yeast could produce high levels of the recombinant HCV E1E2 protein. The final yield of recombinant E1E2

protein was estimated at 35 mg/l. The recombinant HCV E1E2 protein showed complex glycosylation and could bind to CD81, the putative HCV receptor. The purified protein could efficiently induce anti-E1E2 antibodies in rabbits, which were able to neutralize two kinds of HCV pseudotype particles derived from HCV genotype 1a and 1b, as well as HCV virions derived from HCV genotype 2a (2).

### **Mammalian cells**

For over two decades, different mammalian cell lines have been developed as a source of commercial therapeutic proteins for clinical applications, because of their ability for proper protein folding, assembly and post-translational modification (*e.g.*, the correct glycosylation pattern) [30, 31]. However, high costs of production and potential safety concerns remained a challenge for these systems. In one report, a recombinant CHO cell line expressing HCV E2 was established as a secretory form. The recombinant E2 protein was produced at a high level (~ 7 mg/l) and purified using simple immunoaffinity chromatography up to 4 mg/l. This protein could frequently recognized by E2-specific antibodies in patient sera [26].

## **CONCLUSION**

According to the challenges of hepatitis C treatment, it is important to develop a vaccine to prevent HCV infection. The data showed that the recombinant proteins-based vaccine candidates induce an immune response to a limited number of viral epitopes which are expressed in bacteria, insect, yeast or mammalian cells. However, the yield and safety of the recombinant proteins are two important issues for the use of different expression systems.

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