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Classical swine fever Virus: Viral proteins as virulent factors

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Abstract
Classical swine fever virus is the eminently infectious viral disease of pigs and has wide ranging all over the world. It causes severe economic harm to the entire pig industry globally. The CSFV genome encodes a single large polypeptide which undergoes co- and post-translational modifications to produce four structural (C, Erns, E1, E2) and eight non-structural proteins (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). The CSFV viral proteins are the main virulent factors and involved in virus adsorption, induction of protective immunity, virus spreading, virus-host interactions, gene regulation and expression and pathogenesis. CSFV viral proteins can be used to develop recombinant vaccines against CSFV. In this review, the current knowledge regarding the role of both structural and non-structural glycoproteins are responsible for the CSFV virulence is discussed.

Keywords: classical swine fever virus, envelope protein E2, epitope, virulence, pigs

1. Introduction
Classical Swine Fever (CSF) is also referred as Hog Cholera that severely hinders swine industry worldwide and it is a transboundary viral disease that causes a significant threat to pigs. The first outbreak of CSF was recorded in Ohio, USA in 1833 [1] but some reports suggested that an epizootic resembling CSF was appeared in France in 1822 [2]. In 1944, the first suspected case of CSF observed in Aligarh, India [3]. CSFV infection is very challenging to diagnose due to the wide range and non-specific disease symptoms. Though many countries adopted a several non-vaccination eradication policy, vaccination with attenuated vaccines such as e Lapinized Philippines Coronel (LPC) [4] and Hog Cholera Lapinized virus (HCLV), also called as Chinese vaccine strain(C-strain) has been implemented for control of CSF since many years [5]. But CSF remains widespread all over the world and cause severe economic losses due to imposition of trade restrictions on pigs.

2. Virus characteristics
The causative agent of CSF is virus that belongs to the genus *Pestivirus* with in the *Flaviviridae* family. CSFV is closely related to other members of the genus such as *Bovine viral diarrhoea virus* (BVDV) and *Border disease virus* (BDV) [6].

3. Genetic Diversity
CSFV is a single stranded positive sense RNA virus with approximately 12.3 kb in length. Based on phylogenetic and genomic sequence analysis of CSFV classified into one serotype and three genotypes with 10 sub genotypes like 1.1, 1.2, 1.3; 2.1, 2.2, 2.3 and 3.1, 3.2, 3.3, 3.4 [7]. Further sub genotype 2.1 divided into 2.1a, 2.1b, 2.1c and 2.1d and reported to be epidemic in many parts of the world [8, 9]. Sub genotype one again form a separate novel subgenotype that is 1.4 [10]. In India CSFV strains of sub genotype 1.1, 2.2, 2.1 are predominant in domestic pigs of North-Eastern India, wild pigs of North-Eastern India and wild boars in rest of the India respectively [11].

4. Transmission
CSFV is transmitted through two modes of transmission, direct and indirect transmission and the most efficient way of transmission is through direct transmission, it means direct contact (pig to pig) and it may transmit the virus from sows to their offsprings [12]. Indirect transmission (using one or more intermediate steps) is considered it may include swill feeding, livestock trucks contaminated with excretions and secretions of infected pigs, by humans like veterinarians, inseminators, pig dealers, screening teams etc may carry the virus by contaminated clothing and materials [13]. The airborne transmission of virus is possible, where large number of infected pigs found [14]. CSFV may transmitted through pork and pork products because survival of CSFV in meat is very efficient, it may occur up to years when meat stored in frozen condition [15]. It can also be transmitted through artificial insemination with contaminated semen [16]. Some studies reported that CSFV transmitted by birds, pets, rodents and a mosquito is possible but it remains unclear [17, 18].

5. Virus organization
CSFV is a spherical/icosahedral shaped virus particle of 40–60 nm in diameter, consisting of positive sense single stranded RNA genome ~ 12.3 kb in length [19]. CSFV genome contains one long open reading frame (ORF) encodes polyprotein of 3898 aminoacids which is subsequently processed by cellular and viral proteases to

yield 435-kDa protein in which four structural proteins, namely the core protein (C), and three envelope glycoproteins E1, E2, and E^{rns} and eight non-structural proteins such as Npro, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B as shown in fig 1 [20, 21]. The ORF flanked by two untranslated regions at 5' and 3' site, which are highly conserved among all virus isolates [22].

5.1 Structural proteins

5.1.1 C protein

The nucleocapsid (core) protein is a small protein which contains many basic amino acids (lysine and arginine) and its play a major role as a viral structural protein and regulator in gene expression [23].

5.1.2 Erns protein

Glycoprotein Erns is encoded by 226 amino acids and resulting a 41-44 kDa protein. It is involved in the infection process and its RNase activity is probably involved in viral replication and pathogenesis [24]. Two short homologous regions of 8 amino acids in the sequence of Erns, located in the N-terminal half of the protein, are responsible for the RNase activity [25]. It is considered to be the second glycoprotein, responsible for the protective immunity against CSF [26].

5.1.3 E1 protein

E1 protein containing 195 amino acids encodes 33 kDa protein and is the smallest envelope and type I trans-membrane proteins with an N-terminal ectodomain and a C-terminal hydrophobic anchor, which is responsible for CSFV entry to the host cells [27].

5.1.4 E2 protein: An important CSFV structural envelope glycoprotein

Envelope protein E2 (373 amino acids, 51-55KDa) is an important structural glycoprotein which is displayed as homodimer on the outer surface of the CSFV and considered as an important target for induction of the immune responses during infection. It is a major determinant of virulence which is required for cell attachment, virus replication and infection [28]. It forms two types of disulfide-linked dimers, E2-E2 homodimer and E2-E1 heterodimer, which are found on the virion surface. During virus assembly, E2 homodimers are formed first, whereas E1-E2 heterodimers are formed later after the release of E1 from the endoplasmic reticulum chaperone calnexin [29].

5.1.5 Antigenic properties of E2 glycoprotein

CSFV E2 structural protein is the most antigenic, can eliciting neutralizing antibodies in infected animals and induces protective immunity in pigs [30, 31]. Based on competitive binding and antigen capture assay, E2 can be divided into four antigenic domains, A–D, have been identified on the N-terminal half of E2 and constitute two independent antigenic units (in the order of domains B/C and D/A). The antigenic unit of B/C domains is linked by a disulfide bond between ^{693}C and ^{737}C, and other unit of D/A domains is linked by two putative disulfide bonds, one between ^{792}C and ^{856}C and the other between ^{818}C and ^{828}C [32]. The B/C domains are responsible for antigenic specificity among various CSFVs, and the D/A domains of various CSFVs are relatively conserved [33]. It contains a discrete epitope, a novel virulence determinant (TAVSPTTLR, residues 829–837 of CSFV polyprotein) play a major role in CSFV virulence [34] and E2 is responsible for more viral spreading by substitutions in aminoacid residues at T830A position in E2 using site directed mutagenesis [35]. CSFV specific immunodominant neutralizing epitope of the E2 protein is a linear epitope with the SPTTLR as the major binding motif, which is highly conserved among CSFV strains and used as important target for development of peptide-based vaccine [36].

5.2 Non-structural proteins

5.2.1 Npro protein

The first protein encoded by the ORF of CSFV is the nonstructural protein N-terminal protease (Npro) consists of 168 amino acids encoded by 23KDa protein [37]. It cleaves itself autocatalytically into N terminus of the adjacent viral capsid protein C [38]. Npro is responsible for the innate immune response at local sites of virus replication and contributes to pathogenicity of CSFV [39].

5.2.2 P7 protein

CSFV p7 is a small hydrophobic polypeptide with the size of 6-7 kDa is considered as viroporin clearly involved in invitro CSFV replication and responsible for CSFV virulence in pig [40]. It is modulate viral protein interactions and infectious virus production without influencing viral RNA replication [41].

5.2.3 NS2 protein

NS2 is a transmembrane protein, located between p7 and NS3 protein of CSFV which contains an auto-protease responsible for cis-cleavage at the NS2/3 site. Some experiments shows that muted conserved residues within the N- terminus of NS2 protein affected viral RNA replication and decreased virus titer. So that NS2 play a major role in modulating viral RNA replication [42].

5.2.4 NS3 protein

NS3 is a multifunctional protein possessing serine protease, RNA helicase and nucleoside triphosphatase (NTPase) activities. The N-terminal of the protein NS3 act as a protease to process the viral polyprotein and C terminal end of NS3 possess both helicase and NTPase activities. NS3 protein is also involved in viral replication [43].

5.2.5 NS4A and NS4B proteins

NS4A is an 8 kDa protein, consisting of 64 amino acids and it is located in the cell nucleus, cytoplasm, endoplasmic reticulum (ER) and mitochondria. CSFV NS4A induced IL-8 (interleukin-8, a chemokine and activator of neutrophils) production through enhancing MAVS (Mitochondrial antiviral signaling protein) pathway and regulates CSFV replication [44]. The nonstructural protein NS4B is a 38 kDa intracellular membraneprotein which is involved in viral replication. N-terminal domain of NS4B can able to cause CSF viral infectious in pigs [45]. Some residues means amino acid substitutions in NS4B of CSFV to influence virus replication efficiency in vitro [46].

5.2.6 NS 5A and NS5B proteins

The CSFV non-structural protein 5A (NS5A) consists 497 amino acids and is an important component of the

replication system. NS5A comprises the conserved region C2717–C2740–C2742–C2767, which leads to zinc-binding motif that is essential for growth and RNA synthesis of the virus. NS5A synchronize viral RNA replication through binding to NS5B and 3'-UTR [47].

The NS5B protein is a RNA dependent RNA polymerase which is involved in viral replication. CSFV NS5B transcribes the viral genome into minus strands which areused as templates for the production of plus-strand RNA for packaging into viral particles [48].

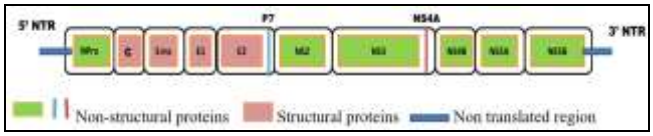

Fig 1: Genome organization of Classical Swine Fever Virus

6. Acknowledgement

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