Virology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Virology

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

Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function

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ARTICLE INFO

Keywords:
Coronavirus
Feline coronavirus
Feline infectious peritonitis
Spike protein
Cleavage activation
Tropism
Pathogenesis
Serotype
Spike structure

ABSTRACT

Feline coronavirus (FCoV) is an etiological agent that causes a benign enteric illness and the fatal systemic disease feline infectious peritonitis (FIP). The FCoV spike (S) protein is considered the viral regulator for binding and entry to the cell. This protein is also involved in FCoV tropism and virulence, as well as in the switch from enteric disease to FIP. This regulation is carried out by spike's major functions: receptor binding and virus-cell membrane fusion. In this review, we address important aspects in FCoV genetics, replication and pathogenesis, focusing on the role of S. To better understand this, FCoV S protein models were constructed, based on the human coronavirus NL63 (HCoV-NL63) S structure. We describe the specific structural characteristics of the FCoV S, in comparison with other coronavirus spikes. We also revise the biochemical events needed for FCoV S activation and its relation to the structural features of the protein.

1. Introduction and historical aspects

Feline coronavirus (FCoV) is the etiological agent of severe disease in domestic and wild felids, known as feline infectious peritonitis (FIP). The disease was first reported in the early 1960s by Holzworth, who included it in the manuscript: “Some important disorders of cats” (Holzworth, 1963). However the viral etiology was only suggested until 1966, when experimental infections of healthy animals were performed using tissues from infected cats (Wolfe and Griesemer, 1966). Viral etiology was finally confirmed in 1968 (Ward et al., 1968; Zook et al., 1968). Two different clinical forms, biotypes or pathotypes have been described for the clinical forms of FCoV (Kipar and Meli, 2014). The first, feline enteric coronavirus (FECV), is a characterized by a mild infection of the enteric tract and is considered ubiquitous in most healthy cats (Pedersen, 2009). The second, feline infectious peritonitis virus (FIPV), is considered the virulent pathotype of FCoV and, in contrast to FECV, the disease caused by FIPV is almost always lethal. However only a relatively small percentage of cats will develop FIP (Brown et al., 2009; Foley et al., 1997).

The virus was first isolated in 1976 using autochthonous peritoneal cells (Pedersen, 1976), then propagated in cell culture using *Felis catus* kidney cells (CRFK). This first isolated strain was named “TN-406” and later known as “FIPV I Black” (Black, 1980; Pedersen et al., 1981a). However, isolation and growing of FCoV has always been difficult, resulting in only few cell culture adapted strains available. Within those strains, WSU 79-1146 and WSU 79-1683 (later known as FECV II 79-1683 and FIPV II 79-1146, respectively) were reported in 1987 and since their isolation they have served as models for the study of FCoV in vitro (McKeirnan et al., 1987). Several studies have been carried out in an attempt to understand the evolution and emergence of FCoV (Chang et al., 2011; Pedersen et al., 1978; Ward, 1970). The first evidence that FCoV was related to other coronaviruses (CoVs) was reported by Ward in 1970. Using electron microscopy, he observed viral particles in tissues from animals infected with an FIP virus and described similarities between these particles and the previously reported human coronavirus 229E and mouse hepatitis virus (Ward, 1970). Following this finding, the relationship between FCoV and other animal coronaviruses (e.g. dogs and pigs) was reported (Pedersen et al., 1978), which served as a starting point for subsequent studies that addressed the close relation between some FCoV and canine coronavirus (CCoV) (Herrewegh et al., 1998). Nevertheless, emergence of FCoV during the last decades is not only related to the FCoV evolution alongside other CoVs, but also to the specific behavioral characteristics of the virus (i.e. switching from FECV to FIPV), and external factors as the increasing popularity of cats as pets, deriving from new and sometimes more intensive breeding methods that favor viral circulation in cat populations (Pedersen, 2009).

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https://doi.org/10.1016/j.virol.2017.12.027
Received 16 October 2017; Received in revised form 22 December 2017; Accepted 23 December 2017

2. Taxonomy, morphology and genetics

FCoV (known as *Alphacoronavirus 1*), belongs to the *Alphacoronavirus* genus (Order: *Nidovirales*, Subfamily: *Coronavirinae*, Family: *Coronaviridae*).[1] Members of the *Coronaviridae* family are grouped in four genera, where *Alphacoronavirus* and *Betacoronavirus* include viruses that principally infect mammals, and are derived from the bat gene pool; whereas *Gammacoronavirus* and *Deltacoronavirus* group viruses that infect birds and mammals and are derived from the avian and pig gene pool (Table 1) (Woo et al., 2012). The viral structure is composed by the nucleocapsid (containing the viral genome) and the outer envelope (Fig. 1A). These two elements stabilize and protect the RNA genome of the virus (Masters and Perlman, 2013). FCoV virions are usually spherical with a moderate level of pleomorphism, with a size range between 80 and 120 nm and club-like surface projections or spikes about 12–24 nm that give the virus its crown-like appearance from where the coronavirus name is derived (Fig. 1A) (Barcena et al., 2009; Fehr and Perlman, 2015). Inside the outer envelope, a helically symmetrical nucleocapsid is found protecting the viral genome, a 5' capped and 3' poly-A tailed single-stranded positive sense RNA (ssRNA +) approximately ~ 29 kilobases (kb) in length (Kipar and Meli, 2014; Masters and Perlman, 2013). The FCoV genome has 11 open reading frames (ORFs) encoding four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), and seven non-structural proteins: the accessory proteins 3a, 3b, 3c, 7a and 7b, and the replicases 1a and 1b (Fig. 1B) (Dye and Siddell, 2005; Pedersen, 2009).

The viral helical nucleocapsid is composed of multiple copies of the RNA binding protein N, a 50 kDa protein which is composed of two domains (NTD and CTD) both with the same affinity to bind RNA, but through different mechanisms. However, both domains are necessary for N binding to the viral RNA (Chang et al., 2006; Hurst et al., 2009). In fact, binding to the viral RNA instead of non-viral RNA is suggested to be enhanced by phosphorylation of the N protein, which induces the specific structural conformation to accomplish this function (Fehr and Perlman, 2015). The most important function of N is to bind and protect the viral genomic RNA (Fig. 1A); however, unlike other viral nucleocapsid proteins (e.g. rhabdoviruses and paramyxoviruses), FCoV (and most coronaviruses) N protein is inefficient at protecting the RNA from ribonuclease activity (Masters and Perlman, 2013; Olsen, 1993). In addition, N protein has been shown to induce cell-mediated immunity, which suggests a possible role in vaccine response studies (Hohdatsu et al., 2003). The mechanism through N can induce immunity is not known, but evidence of interaction between N and M proteins (as well as between N and the nsp3 component of the replicase complex) has been described, and could be related to this feature (Hurst et al., 2013; Sturman et al., 1980).

The FCoV envelope is composed of four main elements: a lipid bilayer derived from the host cell endoplasmic reticulum – Golgi intermediate compartment (ERGIC), and viral proteins E, M and S (Fig. 1A). M is the most abundant structural protein in the virus (Masters and Perlman, 2013). This medium-sized (about ~ 25 to 30 kDa) N-linked glycosylated protein is randomly distributed along the viral envelope, anchored through three transmembrane domains (Armstrong et al., 1984). M has an extensive C-terminal endodomain; and a small ectodomain (about 10% of the N-terminal portion) which makes it less antigenic despite its high abundance (Neuman et al., 2011; Olsen, 1993). The M protein is translated and inserted into endoplasmic reticulum (ER) for viral assembly, but no specific signaling for ER retention has been found in the protein (Fehr and Perlman, 2015). Nevertheless, the M protein interaction with the nucleocapsid, as well as its function in viral membrane remodeling during CoV assembly at the ERGIC, has been described (Neuman et al., 2011).

Table 1
Genus and species from *Coronavirinae* subfamily.

Genus	Species	Viruses
Alphacoronavirus	*Alphacoronavirus 1*	*Transmissible gastroenteritis virus (TEGV)*
		Feline coronavirus (FCoV)
	Porcine epidemic diarrhea virus (PEDV)	
	Human coronavirus NL63 (HCoV-NL63)	
	Human coronavirus 229E (HCoV-229E)	
Betacoronavirus	*Betacoronavirus 1*	*Bovine coronavirus (BCoV)*
		Human coronavirus OC43 (HCoV-OC43)
	Middle East respiratory syndrome-related coronavirus (MERS-CoV)	
	Murine coronavirus	*Mouse hepatitis virus (MHV)*
	Severe acute respiratory syndrome-related coronavirus (SARS-CoV)	
	Human coronavirus HKU1 (HCoV-HKU1)	
Gammacoronavirus	*Avian coronavirus*	*Infectious bronchitis virus (IBV)*
		Turkey coronavirus
	Beluga whale coronavirus SW1	
Deltacoronavirus	*Coronavirus HKU15 (also known as porcine coronavirus PCoV-HKU15)*	

Most representative species of *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*.

The small E protein (~ 8 to 12 kDa) is a type III membrane protein and is also inserted in the viral envelope but is present at a much lesser extent than M or S (Masters and Perlman, 2013). Both E protein C-terminal endodomain and N-terminal ectodomain have been described to have ion channel activity (Fehr and Perlman, 2015; Pervushin et al., 2009). While the function of CoVs E protein (including FCoV) has been associated with assembly at the ERGIC compartment (Corse and Machamer, 2002; Kipar and Meli, 2014), several studies have shown that unlike other CoV structural proteins, viruses with deletions or inactivation of the E protein are less virulent, raising the question of the importance of this protein in viral fitness (Pervushin et al., 2009). However, this specific feature is virus type dependent (DeDiego et al., 2007). The role of the E protein in FCoV replication and pathogenesis has not been studied yet. However, in a study performed in the CoV severe acute respiratory syndrome (SARS-CoV), the authors demonstrated that E ion exchange function in the viral membrane is relevant for viral pathogenesis, as mice infected with mutated or knocked down E protein presented less clinical signs and recovered after infection, while the ones infected with wild type virus did not survive. Nevertheless, the defective E protein did not affect the viral replication (Nieto-Torres et al., 2014).

Among the viral structural proteins, S could be considered the most important in terms of FCoV pathogenesis. The coronavirus S protein is a class I viral fusion protein and is considered the major viral regulator in host cell entry (Bosch et al., 2003; White et al., 2008). Viral fusion proteins are grouped in three different classes according to their structure and biochemical activation processes, where class I proteins are characterized by predominant α-helical secondary structures and a trimeric organization of their pre-fusion and post-fusion state (Harrison, 2013; White et al., 2008). One interesting aspect about class I fusion proteins is the differences in activation of their fusion mechanisms, despite their conserved structure (Millet and Whittaker, 2015; White et al., 2008). However, all fusion proteins in this class undergo major structural changes that allow the viral fusion peptide to contact and anchor into the target cell membrane, and the formation of the "trimer of hairpins" structure followed by the fusion of the outer membranes (hemifusion) and the formation of the fusion pore (White et al., 2008). To successfully induce fusion, a proteolytic activation of the viral protein subunits is often necessary, and this can vary significantly between

[1] Revised on: International Committee on Taxonomy of Viruses (ICTV). Virus Taxonomy: The Classification and Nomenclature of Viruses The Online (10th) Report of the ICTV. Updated: August 2016. https://talk.ictvonline.org/ictv-reports/ictv_online_report/.