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The avian coronavirus spike protein

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ABSTRACT

Avian coronaviruses of the genus *Gammacoronavirus* are represented by infectious bronchitis virus (IBV), the coronavirus of chicken. IBV causes a highly contagious disease affecting the respiratory tract and, depending on the strain, other tissues including the reproductive and urogenital tract. The control of IBV in the field is hampered by the many different strains circulating worldwide and the limited protection across strains due to serotype diversity. This diversity is believed to be due to the amino acid variation in the S1 domain of the major viral attachment protein spike. In the last years, much effort has been undertaken to address the role of the avian coronavirus spike protein in the various steps of the virus' live cycle. Various models have successfully been developed to elucidate the contribution of the spike in binding of the virus to cells, entry of cell culture cells and organ explants, and the *in vivo* tropism and pathogenesis. This review will give an overview of the literature on avian coronavirus spike proteins with particular focus on our recent studies on binding of recombinant soluble spike protein to chicken tissues. With this, we aim to summarize the current understanding on the avian coronavirus spike's contribution to host and tissue predilections, pathogenesis, as well as its role in therapeutic and protective interventions.

1. Introduction

Avian coronaviruses of poultry belong to the genus *Gammacoronavirus* within the order Nidovirales. Avian gammacoronaviruses can cause major health problems with subsequent economic losses in several commercially kept bird species, predominantly chickens (*Gallus gallus*). The genus *Gammacoronavirus* comprises not only viruses of domesticated birds, but also two recently discovered cetacean coronaviruses (Mihindukulasuriya et al., 2008; Woo et al., 2014). In addition, avian coronaviruses belonging to the genera *Gammacoronavirus* and *Deltacoronavirus* have been detected in wild bird species (Chu et al., 2011; Woo et al., 2009, 2012), but details on their pathogenesis and host range are yet unknown. In this review we will focus on the avian gammacoronaviruses of poultry, in particular on the role of the spike protein in the outcome of infection.

The avian infectious bronchitis virus (IBV) causes infectious bronchitis in chickens. It is to date the most important and best-studied *Gammacoronavirus* and is therefore considered the genus' prototype. IBV was the first coronavirus described, and was discovered in the Unites States in the 1930s (Schalk and Hawn, 1931). Currently, it is worldwide present in both industrial and back yard chickens (reviewed by Cook et al., 2012; Jackwood, 2012; Sjaak de Wit et al., 2011). IBV principally infects the epithelium of its hosts' upper airways, which leads to respiratory distress, and predisposes for secondary bacterial airway infections (Dwars et al., 2009; Matthijs et al., 2003). Several IBV strains additionally show a subtype-dependent tropism for other epithelia, including the renal tubuli, the oviduct and parts of the gastrointestinal tract (reviewed in Cook et al., 2012; Ignjatovic and Sapats, 2000; Raj and Jones, 1997). This results in variable morbidity, mortality, pathology and production losses in poultry. The great diversity of IBV strains worldwide makes it difficult to prevent infectious bronchitis in chickens. The presence of IBV-like and other avian coronaviruses in other bird species (including turkey, pheasant, quail, guineafowl, partridge, peafowl, duck, goose and pigeon) (Cavanagh, 2005), complicates the field situation for avian coronaviruses even more.

IBV is an enveloped virus with a positive sense single-stranded RNA genome of 27.6 kb (Masters and Perlman, 2013). The 5′ two-third of the viral genome comprises open reading frame (ORF) 1ab, which encodes for 15 nonstructural replicase proteins (nsp2-16) involved in RNA replication and transcription. The 3′ one-third of the viral genome codes for the structural proteins, which are interspersed by the accessory genes 3a, 3b, 4b/intergenic region, 5a, 5b. These accessory genes are group specific and have, while being dispensable for IBV replication *in vitro* (Casais et al., 2005; Hodgson et al., 2006), yet unknown functions *in vivo*. The structural

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proteins of IBV include the spike protein S, the envelop protein E, the membrane protein M and the nucleocapsid protein N (Masters and Perlman, 2013). After genomic replication, the N protein forms together with the RNA genome the ribonucleocapsid, which is encapsidated by the structural proteins E, M and S to generate the virus particle.

The virus' major adhesion molecule is the spike protein S. The characteristics of the S protein are described in detail in Section 4. Much effort has been undertaken to address the role of the spike protein in various steps of the virus' life cycle, and in the outcome of infection *in vivo* with respect to tropism and pathogenesis. Various models have successfully been developed to study different steps of the IBV infection. This review provides an overview of the literature and recent achievements regarding the spike protein of avian coronaviruses, to summarize our current understanding on the spike's contribution to host and tissue predilections, pathogenesis, and its role in therapeutic and protective interventions.

2. Infectious bronchitis

The disease described as 'infectious bronchitis' is a collection of symptoms caused by IBV subtypes, which can be discriminated based on genotype, serotype and protectotype (Sjaak de Wit et al., 2011). The classical subtype causing respiratory disease, IBV Massachusetts 41 (M41), was isolated by Van Roekel in the United States in 1941 (reviewed by Fabricant, 1998). Subtypes other than M41 also cause respiratory disease, but with varying severity. Respiratory disease is often clinically characterized by dyspnea, coughing, rales and serous nasal discharge (Cavanagh and Gelb, 2008). It is caused by infection of the ciliated epithelium of the upper respiratory tract (mainly nasal cavity and trachea), resulting in loss of ciliary activity, degeneration, desquamation and loss of these cells. In addition, infected tissues shows hyperemia and inflammation (Fig. 1A), which is mainly characterized by the presence of heterophilic granulocytes and lymphocytes (Fig. 1B). IBV can also spread to the lower respiratory tract and cause aerosacculitis (Bezuidenhout et al., 2011). Usually, the epithelium is restored to normal within 2–3 weeks *via* a state of extensive hyperplasia (Dwars et al., 2009; Nakamura et al., 1991; Purcell and McFerran, 1972).

From the respiratory tract, the virus spreads through the host *via* viremia (Jones and Jordan, 1972) to the epithelial cells of the renal tubuli (Chen and Itakura, 1996; Condron and Marshall, 1986; Purcell et al., 1976) and the ciliated epithelium of the oviduct (Crinion et al., 1971; Jones and Jordan, 1971). Here the virus causes respectively renal failure with urate obstruction due to tubular necrosis with mononuclear inflammation (Chen and Itakura, 1997; Jones, 1974), and oviductal necrosis and malformation leading to abnormal egg production and inability to lay (Chousalkar and Roberts, 2007). The severity of the disease in various organs depends on the IBV subtype and ultimately determines the mortality in chickens.

Minor pathological changes due to IBV infection can occasionally be seen in other organs. The virus has been shown to infect glandular epithelial cells of the proventriculus (Yu et al., 2001), as well as cells resembling histiocytes and lymphoid cells in and enterocytes covering the cecal tonsils (Owen et al., 1991). However, this does not result in significant clinical gastrointestinal disease. IBV can also infect the Harderian gland (Toro et al., 1996, 1997; van Ginkel et al., 2008), an organ involved in the immune response. Finally, it has been reported that testicles can be infected, from which IBV can be venereally transmitted by the semen (Gallardo et al., 2011).

Avian coronaviruses have been detected in various other poultry species. While some of these IBV- or IBV-like viruses display high sequence similarity to IBV or IBV vaccine strains (Liu et al., 2005; Sun et al., 2007), others strains are much more divergent and may represent different virus species (reviewed in Cavanagh, 2005). For example, turkey coronavirus TCoV is very divergent in its spike gene, and causes in contrast to the respiratory disease observed for IBV, gastrointestinal disease in turkeys (*Meleagris*

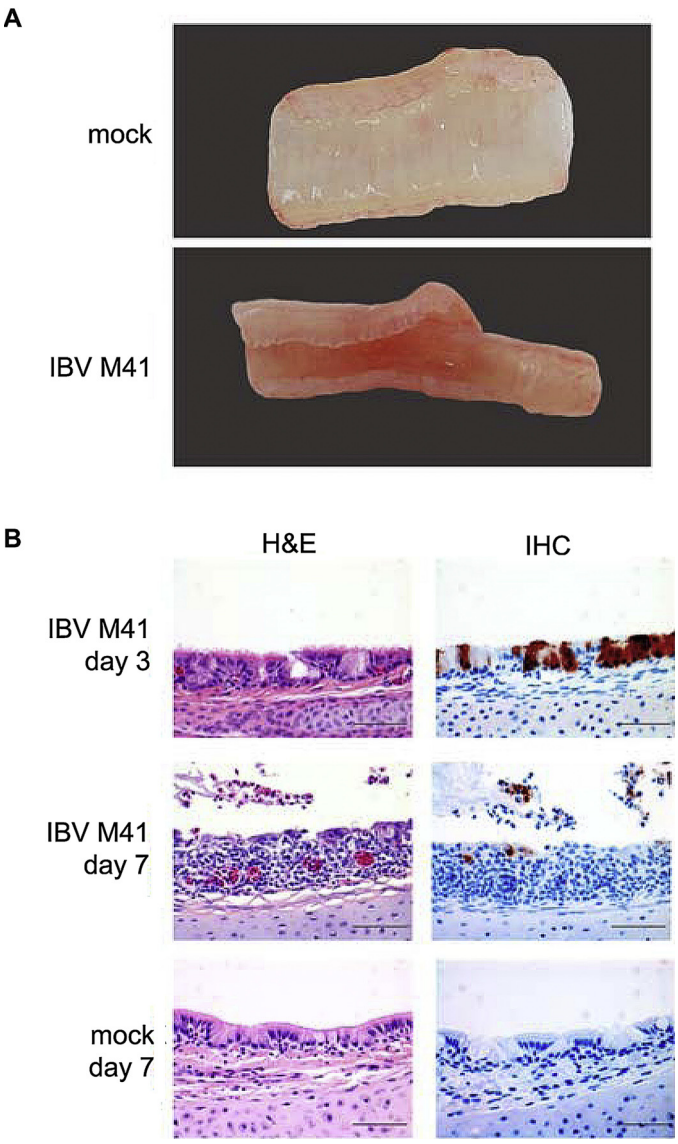

Fig. 1. Macroscopic, histological and immunohistochemical analyses of chicken trachea of mock-infected or IBV M41-infected layer chickens. Seven-day-old SPF layer chickens were oronasally infected with PBS (mock) or M41 and sacrificed at various time points after infection. (A) Longitudinally opened trachea of mock (upper) and IBV-M41 infected (lower) chicken at 7 dpi; the M41-infected trachea shows small amounts of mucoid material in the lumen and marked multifocal hyperemia of the mucosa. (B) Hematoxylin and eosin (H&E) (left) and anti-IBV S2 MAb 48.4 immunohistochemistry staining (right) of a section of the trachea of a mock-infected (7 dpi) or M41-infected (3 dpi and 7 dpi) chicken. The trachea of the M41-infected chicken at 3 dpi shows an intact epithelial lining with minimal hyperemia, while both ciliated epithelial cells and non-ciliated mucus-producing epithelial cells show marked intracytoplasmic presence of S2 antigen. At 7 dpi, the trachea has lost normal architecture due to desquamation of the ciliated and non-ciliated epithelium with replacement by a hyperplastic, more squamous non-ciliated epithelium, infiltration by large numbers of lymphocytes, marked hyperemia and in the superficial layer presence of necrotic cells. The lumen contains desquamated epithelial cells, marked numbers of heterophilic granulocytes and abundant mucoid material. Both the epithelial lining and lumen show cells containing the S2 antigen. There are no changes observed in the mock-infected chicken trachea. Scale bars represent 50 μm.