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Review article

Antigenic properties of avian hepatitis E virus capsid protein

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ABSTRACT

Avian hepatitis E virus (HEV) is the main causative agent of big liver and spleen disease and hepatitis-splenomegaly syndrome in chickens, and is genetically and antigenically related to mammalian HEVs. HEV capsid protein contains immunodominant epitopes and induces a protective humoral immune response. A better understanding of the antigenic composition of this protein is critically important for the development of effective vaccine and sensitive and specific serological assays. To date, six linear antigenic domains (I–VI) have been characterized in avian HEV capsid protein and analyzed for their applications in the serological diagnosis and vaccine design. Domains I and V induce strong immune response in chickens and are common to avian, human, and swine HEVs, indicating that the shared epitopes hampering differential diagnosis of avian HEV infection. Domains III and IV are not immunodominant and elicit a weak immune response. Domain VI, located in the N-terminal region of the capsid protein, can also trigger an intense immune response, but the anti-domain VI antibodies are transient. The protection analysis showed that the truncated capsid protein containing the C-terminal 268 amino acid residues expressed by the bacterial system can provide protective immunity against avian HEV infection in chickens. However, the synthetic peptides incorporating the different linear antigenic domains (I–VI) and epitopes are non-protective. The antigenic composition of avian HEV capsid protein is altogether complex. To develop an effective vaccine and accurate serological diagnostic methods, more conformational antigenic domains or epitopes are to be characterized in detail.

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1. Introduction

Avian hepatitis E virus (HEV) is the main causative agent of big liver and spleen disease and hepatitis-splenomegaly syndrome in chickens (Haqshenas et al., 2001; Payne et al., 1999). The virus infection can cause a decreased egg production, a gradually elevated mortality rate in laying and broiler breeder hens aged

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from 30 to 72 weeks (Clarke et al., 1990; Handlinger and Williams, 1988). Recently, it was reported that a subclinical infection in chickens can also be caused by avian HEV infection (Peralta et al., 2009; Sun et al., 2004). To date, many avian HEV isolates have been characterized in different countries (Bilic et al., 2009; Billam et al., 2007; Haqshenas et al., 2001; Hsu and Tsai, 2014; Kwon et al., 2012; Morrow et al., 2008; Zhao et al., 2010).

Avian HEV, belonging to the genus *Orthohepevirus*, is classified as a separate, floating species in the Hepeviridae family (Smith et al., 2014) and is a single-stranded, positive sense RNA virus. The complete genome of the virus is approximately 6.6 kb, which is 600 base pairs (bp) smaller than those of swine and human HEVs, and consists of three open reading frames (ORFs), ORF1, ORF2, and ORF3 (Huang et al., 2004). The ORF1 encodes a viral non-structural protein with multiple functional domains; the ORF2 encodes the capsid protein, while the smallest one, ORF3, encodes a small protein possessing regulatory properties (Huang et al., 2004). So far, at least four different genotypes of avian HEV from chickens have been identified worldwide; genotype 1 in Australia and Korea, genotype 2 in the USA, genotype 3 in Europe and China, and genotype 4 in Hungary and Taiwan (Banyai et al., 2012; Bilic et al., 2009; Haqshenas et al., 2001; Hsu and Tsai, 2014; Kwon et al., 2012; Marek et al., 2010; Zhao et al., 2010). However, all genotypes avian HEV represent a single serotype, which facilitates the development of diagnostic assays for detecting anti-avian HEV antibodies (Meng, 2010).

The characterization of antigenic domains and epitopes present on the surface of viral particles is helpful in developing immunoassays and vaccines. The capsid protein of avian HEV contains immunodominant epitopes of viral particles and is responsible for the induction of a protective humoral immune response (Dong et al., 2011; Guo et al., 2007; Guo et al., 2006; Haqshenas et al., 2002; Zhou et al., 2008). Therefore, understanding the antigenic composition of this protein has remarkable implications in the field of diagnostics and the development of a candidate vaccine for avian HEV. Over the last decade, substantial progress has been achieved in avian HEV research: exploration has been conducted about the antigenic domains, epitopes, and neutralizing epitopes of avian HEV capsid protein by using synthetic peptides and expressed recombinant proteins (Guo et al., 2006; Haqshenas et al., 2002; Wang et al., 2014; Wang et al., 2015). In the meantime, the serological diagnostic assays and subunit vaccines have also been developed through the utilization of the different antigenic domains as targets in the laboratory examinations (Huang et al., 2002; Zhao et al., 2013). However, due to the lack of efficient culture system in vitro and the antigenic complexity of avian HEV, refining the serological assays remains a challenge. Few ELISA kits have been developed, but no vaccine has been commercialized so far. In this review, updated information is pooled from some previous studies regarding the antigenic characterization of avian HEV capsid protein. Furthermore, this review focuses on understanding the composition, antigenicity and neutralization of the antigenic domain in a systemic manner to facilitate the development of the most reliable and accurate serological assay methods and to contribute to designing a suitable candidate vaccine.

2. Analysis of amino acid sequences and computer predictions

The capsid protein of avian HEV shares above 98% amino acid sequence identities among different genotypes (1–4), and 48–49% amino acid sequence identities with the capsid protein of human and swine HEVs (Haqshenas et al., 2002; Huang et al., 2004). This similarity suggests that avian HEV has only a single serotype in different genotypic strains and is antigenically related to human and swine HEVs. By using the Kyte-Doolittle, Jameson-Wolf and Emini methods to analyze the hydrophilicity, antigenicity, and surface probability plots of avian HEV capsid protein, six hydrophilic, antigenic, and surface potential regions were predicted at different amino acid (aa) positions of the protein: 18–48, 112–133, 191–235, 387–405, 506–515, and 582–606 (Fig. 1). However, further studies are needed to confirm that these regions are the real antigenic domains existing on the avian HEV particle.

3. Antigenic domains or epitopes composition

Similarly to the methods for identifying antigenic domains of human HEV capsid protein, the antigenic domains or epitopes of avian HEV capsid proteins were also identified by using synthesized peptides and expressed truncated recombinant proteins. In an early study, four antigenic domains (I–IV) were predicted at aa 389–410, aa 461–492, aa 556–566, and aa 583–600 in the avian HEV capsid protein via the Welling method (Fig. 2) (Haqshenas et al., 2002). Subsequently, B-cell epitopes were determined in the aforementioned four antigenic domains through indirect ELISA with nine synthesized peptides used as antigens and the corresponding rabbit anti-peptide antisera and convalescent chicken antisera utilized as antibodies (Guo et al., 2006). The results showed that all four predicted antigenic domains reacted with the convalescent chicken antisera, indicating that when infected by avian HEV, the chickens can elicit immune response to these four antigenic domains. Furthermore, studies on the cross-reactivity between 9 peptides and their respective anti-peptide sera revealed that a B-cell epitope is located in domain I between aa 399 and 410, one or more B-cell epitopes are located in domain II between aa 473 and 492, and the N-terminal amino acid residues (aa 461–473) block the C-terminal epitope presentation in domain II (Guo et al., 2006). In addition, two neutralization epitopes situated in aa 476–513 and aa 513–570 of the capsid protein were

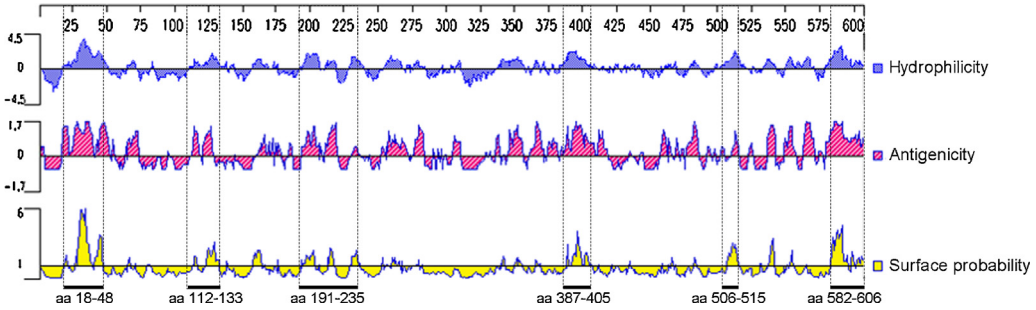

Fig. 1. Hydrophilicity plot, antigenic index, and surface probability were predicted by DNASTAR software by using the methods described by Kyte–Doolittle, Jameson–Wolf and Emini, respectively. Based on the analysis results, six antigenic domains were predicted and separately located at amino acid (aa) 18–48, aa 112–133, aa 191–235, aa 387–405, aa 506–515, and aa 582–606.