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Review

The antigenic architecture of the hemagglutinin of influenza H5N1 viruses

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ABSTRACT

Human infection with the highly pathogenic avian influenza A virus H5N1 is associated with a high mortality and morbidity. H5N1 continues to transmit from poultry to the human population, raising serious concerns about its pandemic potential. Current influenza H5N1 vaccines are based upon the elicitation of a neutralizing antibody (Ab) response against the major epitope regions of the viral surface glycoprotein, hemagglutinin (HA). However, antigenic drift mutations in immune-dominant regions on the HA structure allow the virus to escape Ab neutralization. Epitope mapping using neutralizing monoclonal antibodies (mAb) helps define mechanisms of antigenic drift, neutralizing escape and can facilitate pre-pandemic vaccine design. This review explores the current knowledge base of the antigenic sites of the H5N1 HA molecule. The relationship between the epitope architecture of the H5N1 HA, antigenic evolution of the different H5N1 lineages and the antigenic complexity of the H5N1 virus lineages that constitute potential pandemic strains are discussed in detail.

1. Introduction

1.1. Human infection with H5N1 influenza

In Hong Kong in 1997, the H5N1 subtype avian influenza A virus crossed the species barrier directly from poultry to humans (de Jong et al., 1997; Gambotto et al., 2008; Subbarao et al., 1998). The current fatality rate from circulating H5N1 strains is around 59% (Dudley, 2009; Kayali et al., 2011). The precursor virus A/goose/Guangdong/1/96 was isolated in the Guangdong province of China in 1996 (Xu et al., 1999). Post 1997, avian H5N1 influenza A virus outbreaks have been reported in poultry farms and markets in several East Asian countries including Vietnam, Thailand, Indonesia, Japan, and Cambodia (Webster and Govorkova, 2006; Webster et al., 2005, 2007). H5N1 is now endemic in poultry in these countries and has also spread to wild bird populations (Chen et al., 2006; Komar and Olsen, 2008; Wibawa et al., 2011). More recently, the virus has spread to avian populations across Europe and emerged in Turkey, Russia, and Africa (Webster and Govorkova, 2006). These events show that H5N1 viruses currently circulating in poultry and wild-aquatic birds have the potential to cause serious illness in humans without re-assortment with human influenza viruses or the need for an intermediate mammalian host (Nguyen et al., 2005; Smith et al., 2009; Wallace et al., 2007; Wan et al., 2008).

Empirical evidence suggests that the antigenic evolution of the influenza virus as it escapes neutralizing Abs involves transitions from one antigenic cluster to another (Ndifon et al., 2009; Smith et al., 2004). The sustained circulation of H5N1 has lead to the evolution of distinct viral lineages which show significant antigenic variation in the HA sequences (Ducatez et al., 2011; Group, 2008; Webster and Govorkova, 2006; Wu et al., 2008). To date, based on phylogenetic analysis of the HA gene sequences, the H5N1 viruses can be divided into 10 antigenic clades (0–9) (Chen et al.,

Abbreviations: aa, amino acid; CDC, Centre for Disease Control; ELISA, enzyme-linked immunosorbant assay; FDA, Federal Drug Administration; GFPDL, Genome Fragment Phage Display Libraries; HA, hemagglutinin; HI, hemagglutination inhibition; HAMA, human anti-mouse-antibody; mAbs, monoclonal antibodies; NA, neuraminidase; RBS, receptor binding site; RPL, random phage display library; SPR, Surface Plasmon Resonance; VLP, virus-like particle; WHO, World Health Organization.

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2006; Ducatez et al., 2011; Group, 2008; Webster and Govorkova, 2006; Wu et al., 2008). Currently, only viruses from clades 0, 1 and 2 have infected humans since the initial 1997 outbreaks (Abdel-Ghafar et al., 2008; Dung Nguyen et al., 2008; Webster and Govorkova, 2006; Xu et al., 1999). The clade 0 viruses were responsible for the 1997 Hong Kong outbreaks (Abdel-Ghafar et al., 2008; Dung Nguyen et al., 2008; Webster and Govorkova, 2006; Xu et al., 1999). The clade 0 viruses are the precursor of the clade 1 and 2 viruses, which also contain sub-lineages (Ducatez et al., 2011; Dung Nguyen et al., 2008). Clade 1 encompasses human and avian isolates from Vietnam, Thailand, Cambodia, Laos and Malaysia (Webster and Govorkova, 2006; Wu et al., 2008). The reemergence of H5N1 in Southeast Asia in 2003–2005 was largely due to the clade 1 viruses (Abdel-Ghafar et al., 2008; Dung Nguyen et al., 2008; Webster and Govorkova, 2006; Xu et al., 1999). The clade 2 viruses are the most diverse and can be further sub-divided into three sub-clades of distinct geographic distribution (Webster and Govorkova, 2006; Wu et al., 2008). Sub-clade 2.1, Indonesia; Subclade 2.2, Europe, Middle East, and Africa (Webster and Govorkova, 2006; Wu et al., 2008) and Subclade 2.3, China, Vietnam and Laos (Webster and Govorkova, 2006; Wu et al., 2008). The three subclades can be further divided into sub-lineages. The clade 2 viruses are largely responsible for the recently reported human H5N1 infections (Webster and Govorkova, 2006; Wu et al., 2008). Anti-serum raised against A/Indonesia/5/05 (clade 2.1) has been shown to cross-react with clade 2.2, 2.3.4 and partially with the clade 1 viruses (Boon and Webby, 2009). Anti-serum against A/Wooper swan/Mongolia/244/05 (clade 2.2) cross-reacts with clade 2.1 viruses but not with clade 1 and 2.3.4 viruses (Boon and Webby, 2009). Anti-sera from the clade 7 viruses does not cross-react with either the clade 1 or 2.34 viruses (Nguyen et al., 2009). A comprehensive serologic study from Zhou et al. (2012) revealed that the H5N1 sub-clades could be grouped into antigenic clusters with clades 0, 1, 3, 4, 5, 6, 7.1 and 9 grouped into antigenic cluster 1; sub-clades 2.2.1, 2.1.3.2, 2.3.4, 2.4, 2.5 and 8 grouped into cluster 2; and sub-clades 2.3.21 and 7.2 were in individual groups. Supplementary Fig. 1 shows a sequence alignment of representative viruses from each clade and a surface map of the sequence variations mapped onto the A/Vietnam/1203/04 HA crystal structure. Clearly, the most variable inter-clade positions are largely located on the membrane-distal globular head, proximal to the receptor binding site (RBS) (Supplementary Fig. 1). Together these findings underscore the genetic divergence and antigenic complexity of the H5N1 virus sub-lineages and the associated difficulties for human vaccine development (Ducatez et al., 2011).

1.2. The H5 hemagglutinin

The influenza viruses belong to the family of orthomyxoviride (Wright and Webster, 2001). The single-stranded negative sense RNA genome consists of 8 segments that encode 11 viral proteins (Wright and Webster, 2001). There are two surface proteins, the HA and the NA and 9 internal proteins (Wright and Webster, 2001). The combinations of HA and NA combinations seen in the known strains of influenza A viruses are comprised of 16 sub-types (H1–H16) and 9 NA subtypes (1–9) (Wright and Webster, 2001). The 16 influenza sub-types can be further divided into two phylogenetic groups; group 1 which includes sub-types H1, H2, H5, H6, H8, H9, H11, H12, H13 and H16; group 2 which includes sub-types H3, H4, H7, H10, H14 and H15 (Air, 1981; Skehel, 2009).

The HA glycoprotein is the principal surface antigen on the influenza virus, and as such is a major target for neutralizing antibodies (Ducatez et al., 2011; Ekiert et al., 2009; Ekiert and Wilson, 2012). Accordingly, pandemic viruses possess an antigenically distinct HA to which the human immune system is naive. The primary function of the HA is to initiate internalization of the virus into the host cell by binding to host sialic acid receptors attached to membrane glycoproteins, glycophospholipids and proteoglycans (Wright and Webster, 2001). Following binding to cell surface receptors the virion enters the cell by endocytosis and is transported to the low pH environment of the endosome (Bullough et al., 1994; Rossman and Lamb, 2011; Skehel, 2009; Skehel and Wiley, 2000; Wilson et al., 1981). At low pH the HA2 undergoes major conformational rearrangements associated with the fusion of the viral and cellular membranes (Bullough et al., 1994; Rossman and Lamb, 2011; Skehel, 2009; Skehel and Wiley, 2000; Wilson et al., 1981). The quaternary structure of the mature influenza HA consists of a trimer of identical HA subunits (Wiley et al., 1981). On a structural level, the H5 HA is not unlike the other HA sub-types (Ha et al., 2002; Yamada et al., 2006; Yang et al., 2007). The precursor HA0 monomer is expressed as a single polypeptide and is processed by host proteases into the two disulfide-linked HA1 and HA2 subunits (Skehel and Wiley, 2000; Wiley et al., 1981). The HA trimer is formed in the endoplasmic reticulum and is transferred to the cellular membrane via the golgi apparatus (Skehel and Wiley, 2000; Wright and Webster, 2001). The HA1 polypeptide encompasses the membrane-distal globular head that harbors the RBS and vestigial esterase domains (Fig. 1) (Skehel and Wiley, 2000; Wilson et al., 1981). The HA2 polypeptide encompasses the stalk region and the CD helix that forms the trimeric coiled-coil and the A-helix (Bullough et al., 1994; Skehel and Wiley, 2000; Wilson et al., 1981).

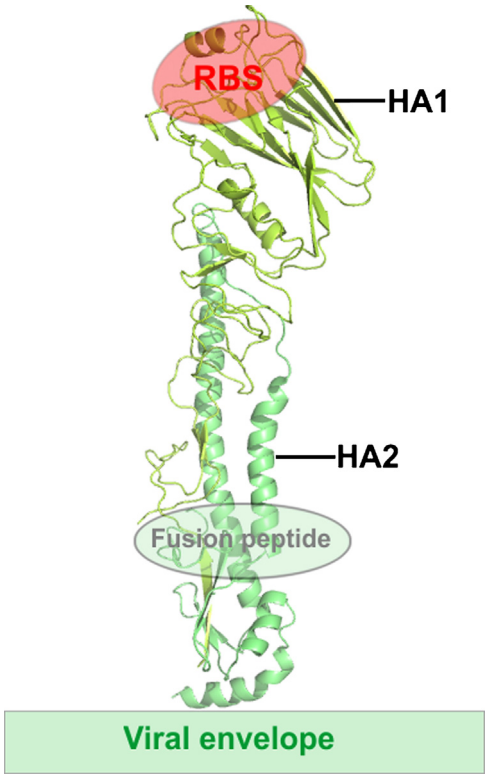

Fig. 1. Crystal structure of the A/Vietnam/1203/04 HA monomer (PBB ID: 2FK0). The sialic acid receptor binding site (RBS) is shaded in red. The HA1 and HA2 polypeptides are indicated, the latter contains the fusion peptide region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

The sialyl-glycan receptors bound by the HA are usually linked to galactose (Gal) in an α2,6 or α2,3 configuration (Gambaryan et al., 1997; Gamblin et al., 2004; Skehel and Wiley, 2000; Stevens et al., 2006c, 2008). In the case of the influenza A viruses, which circulate across a number of different mammalian species, the host glycan distribution and binding specificity of the viral HA is what largely determines the host range of the virus (Couceiro et al., 1993;