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Review

A review of influenza haemagglutinin receptor binding as it relates to pandemic properties

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ABSTRACT

Haemagglutinin is a determinant of many viral properties, and successful adaptation to a human-like form is thought to be an important step toward pandemic influenza emergence. The availability of structurally distinct sialic acid linked receptors in the sites of human and avian influenza infection are generally held to account for the differences observed, but the relevance of other selection pressures has not been elucidated. There is evidence for genetic and structural constraints of haemagglutinin playing a role in restricting haemagglutinin adaptation, and also for differences in the selection pressure to alter binding, specifically when considering virus replication within host compared to transmission between hosts. Understanding which characteristics underlie such adaptations in humans is now possible in greater detail by using glycan arrays. However, results from these assays must also interpreted in context of an as yet still to be determined detailed knowledge of the structural diversity of sialic acids in the human respiratory tract. A clearer understanding of the evolutionary benefits conveyed by different haemagglutinin properties would have substantial impact and would affect the risk we allocate to viral propagation in different species, such as swine and poultry. Relevant to the H5N1 threat, current evidence also suggests that mortality associated with any emergent pandemic from current strains may be reduced if haemagglutinin specificity changes, further emphasising the importance of understanding how and if selection pressures in the human will cause such an alteration.

1. Introduction

The ability to predict the emergence of new pandemic strains is a topical issue. When the World Health Organisation (WHO) declared the H1N1 swine-flu outbreak a pandemic, it became the fourth recorded influenza pandemic in a century to emerge from a strain previously sustained outside the human population. Previous pandemics have consisted of the zoonosis of another subtype of influenza which, due to genetic diversity, is antigenically different from the currently circulating strains. Without prior exposure or anticipatory vaccine cover, the antigenic novelty allows the new strain to transmit rapidly through an immunogenically naive global population, inevitably resulting in increased mortality. This change in influenza subtype or 'antigenic shift' had been the major paradigm for future pandemics. With the exception of the reintroduction of H1N1 in humans in the early 1970s, the new subtype results in the replacement of the previous circulating subtype. The H2 subtype replaced H1 in 1957 and was subsequently replaced by H3 in 1968 [1]. Given the recent circulation of H1N1 in humans, the emergence of swine origin H1N1 in 2009 was unexpected. Although the 2009 pH1N1 strains were antigenically different from the human H1N1 strains, which warranted a novel pH1N1 vaccine, there is evidence of prior immunity in the population [2]. This demonstrates the difficulty in accurately predicting how close any particular strain may actually be to achieving sustained human spread and forces us to reconsider the barriers to avian or swine to human zoonosis.

Currently, the H5 subtype is still of great concern, with the current total number of confirmed human cases at 562 [3], but subtypes H7, H9 and H10 have also been reported in humans [4] and any new subtype could theoretically be the foundation of the next pandemic. Although there have already been suspected cases of human-human transmission of H5N1 [5–7], circumstances are generally exceptional and the transmission has not been sustained [8].

In response to pandemic concerns, structural and genetic properties characteristic of human viral strains are beginning to be defined. A hope is that through the identification of the key features that avian viruses require to achieve pandemic spread, we will be able to better evaluate the true risk posed by current avian strains. Haemagglutinin (HA) has been the focus of a large area of investigation and many people have reviewed the features of HA found

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in viruses that have successfully invaded the human population [9–12]. These properties are being defined in increasing detail and potential explanations for the evolutionary forces that shape these characteristics have been put forward. Rarely, however, has the relevance of each of these evolutionary factors to viral success been discussed in relation to each other. Here we describe the functional significance of HA in pandemic emergence before considering evidence for the existence and relative contribution of a variety of selection pressures imposed upon it. Ways in which more detailed descriptions of these factors are relevant to prediction and prevention concerning pandemic strains are then explored, highlighting avenues for further research.

2. The function and significance of the influenza haemagglutinin protein

Influenza haemagglutinin is a glycoprotein coded in the HA gene segment of the influenza virus and along with neuraminidase, is expressed as a trimer on the surface of the viral capsid. HA allows the recognition of cells in the upper respiratory tract or erythrocytes by binding to glycans containing the monosaccharide sialic acid. The virus is subsequently engulfed by the cell into an endosome and the lower pH of the endosome facilitates a structural rearrangement of HA. HA then fuses with the membrane of the endosome and allows release of the ribonucleoproteins (RNPs) into the cytoplasm. The RNPs are transported into the nucleus followed by transcription, and replication of the viral genome. The HA, along with the other newly generated viral proteins and a replicated genome, is then incorporated into the envelope of the influenza virion as it buds from an infected host cell. The neuraminidase gene of the virus is then required for efficient release of the newly formed virion.

Despite the focus of this review on HA, the relevance of adaptation in other viral proteins as part of the generation of pandemic strains should certainly not be forgotten. Even in the wider context of the whole viral lifecycle however, HA is a uniquely significant component of the virus; structural variations greatly affect viral antigenicity, while its function in cellular attachment through membrane fusion makes it an important determinant of other viral properties including host specificity, replication potential, transmissibility and pathogenicity [13]. Ultimately, HA is involved in a number of steps that may represent many of the most substantial barriers to productive human infections by otherwise avian strains (summarised in Fig. 1).

In seasonal influenza, the most significant currently understood predictor of viral strain success is the extent to which HA differs antigenically from the currently circulating strains. This knowledge has meant that direct measurements of antigenic similarity can be employed to make effective predictions as to which viral strains are likely to propagate most effectively through the global population [14,15]. Even ignoring the exceptionally deadly 1918 pandemic, the last two pandemics of the 20th century caused over one million deaths each [16]. The value of pre-empting the emergence of pandemic strains in a similar way to the efforts with seasonal viral strains is self-evident. To date, however, the properties that contribute to the success of novel avian strains in human hosts are far less clearly defined, but are equally important in our understanding of how new viral strains invade the global population. In this respect, the direct effect HA has upon a number of viral characteristics has made it an important area for investigation.

3. Avian and human receptor specificities

HA has been implicated as a key determinant of host specificity and consequently, adaptation of avian HA to humans was an important stage in the evolution of previous pandemic strains. In the life cycle of the virus, HA functions to bind sialic acids (SA) on host cell surfaces, the first stage in a process that leads to internalisation of the virus and invasion into the cell body. SA are a diverse family of sugars with a nine carbon backbone, which exist in a variety of arrangements, branched and unbranched, and with a range of associated chemical groups. They are typically found at the distal end of glycan chains, which are a feature of all cell types. Consequently, a capability to bind to the kinds of SA present in the human respiratory tract is an important feature of human influenza strains [17].

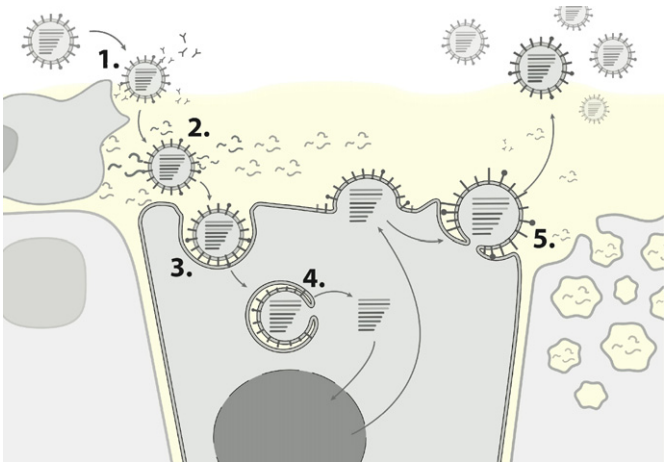

Fig. 1. The role of HA in steps to productive human infection. 1. Evasion of acquired immunity, through novel antigenic features. 2. Avoidance of innate immunity, including possible loss of 'decoy' receptor binding to mucin. 3. Productive SA binding, specifically to α2–6 linkages prevalent in the human upper respiratory tract. 4. Effective membrane fusion. 5. Efficient viral budding, for example through an appropriate "HA/NA balance".

In 1983, Rogers and Paulson first demonstrated that H3 avian viruses showed a significant preference for binding erythrocytes derivatised to contain SA with an α2–3 linkage, compared to those with an α2–6 linkage [18]. In contrast, human isolates were shown to have the reverse preference. Later, this pattern was also identified in larger studies, including studies investigating H1 and H2 subtypes [10,19]. Further results from Paulson and colleagues, which demonstrated an apparent abundance of SA with α2–6 linkages in the human respiratory tract, have often been taken as evidence that avian viruses cannot infect humans because of a paucity of suitable SA on human respiratory cells. Perhaps for this reason it has been commonly assumed that, in terms of HA, adaptation to bind the more abundant α2–6 linkages has been the overriding evolutionary barrier to human infection for avian strains. In support of this hypothesis, experiments have correlated changes in HA receptor preference strongly with changes in transmissibility [20–23]. However, it is always important to consider that it does not *necessarily* follow that binding to α2–6 linkages on mammalian cells is the most significant HA property that has been affected under such modification.

Although widespread, the correlation first observed by Paulson et al. is not absolute and there are examples of avian isolate strains that have been shown to possess a preference for binding SA with α2–6 linkages [24], while equally, some human isolates have retained an α2–3 linkage preference, an example being recent H5N1 viruses isolated from humans [25]. With some qualification, however, α2–3 and α2–6 binding preference has remained an important phenomenon since although α2–3 binders can infect humans in exceptional circumstances, all strains successfully propagating as an epidemic or pandemic in the human population appear to have an α2–6 preference, or at least joint α2–6, α2–3