Infection of the endothelium by influenza viruses

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Summary
Highly pathogenic avian influenza viruses are not only the cause of devastating “bird flu” outbreaks in domestic fowl, but are also occasionally the cause of human disease with a high mortality rate as is being currently observed with the H5N1 viruses in South-East Asia. Infection in birds is systemic with hemorrhages and edema as characteristic symptoms, and virus replication in the endothelium appears to play an important role in pathogenesis. Some of the factors determining endotheliotropism have been elucidated at the molecular and cellular level. They include proteolytic activation of the hemagglutinin, polarity of virus budding, and tissue specific expression of virus receptors.

Keywords
Influenza virus, endotheliotropism, fowl plague, hemorrhages

Influenza viruses have a high genetic variability
Influenza viruses are segmented negative-stranded RNA viruses that are members of the orthomyxovirus family. Among the three genera – A, B, and C – influenza A viruses are the most important ones. The genome of influenza A viruses consists of 8 RNA segments, each of which contains the genetic information for one or two proteins. Genome segmentation facilitating gene reassortment and high mutation rates are the basis for the extraordinary genetic variability of influenza viruses. Virus particles have a helical ribonucleoprotein core formed by the genomic RNA, the nucleocapsid protein, and the polymerase complex that is responsible for RNA replication and transcription. The ribonucleoprotein is surrounded by the viral envelope which is coated on its inner side by the matrix protein and which has 3 integral membrane proteins: the hemagglutinin (HA) and the neuraminidase (NA) forming the surface spikes and an ion channel protein (M2) (Fig. 1). HA mediates virus entry by binding to neuraminic acid receptors on the cell surface and by inducing membrane fusion. The fusion activity depends on proteolytic cleavage of HA by host proteases. After entry and M2 mediated uncoating, the genome is transported into the nucleus where transcription and RNA replication take place. Ribonucleoprotein is assembled in the nucleus and then exported into the cytoplasm. Envelope proteins are translated in the rough endoplasmic reticulum and processed during transport to the plasma membrane where virus particles are assembled in a budding process. Removal of receptors from the surface of infected cells by NA results in virus release.

Influenza A viruses have a wide host range
Influenza A viruses are found in humans, pigs, and several other mammals as well as in many birds. It is generally believed that the mammalian viruses emerge from the large reservoir of avian strains comprising 16 HA and 9 NA subtypes (1, 2). This includes the human influenza viruses causing pandemics in 1918, 1957, 1968, and 1977 that have been derived from avian viruses either by gene reassortment or by transmission of the entire genome (Fig. 2). Most influenza A viruses cause local infection that is confined to the respiratory tract or, in the case of avian strains, to the gut. The avian strains showing this type of infection usually have low pathogenicity or are completely apathogenic. In contrast, some avian strains belonging to subtypes H5 and H7 cause generalized infection. These viruses are highly pathogenic, killing the birds within a few days. For decades, the disease has been known under the name fowl plague, but is now frequently called bird flu. An important determinant for the differential infection spread of highly pathogenic and low pathogenic avian influenza viruses is cleavage activation of HA, which, in the case of the pathogenic strains, is exerted by ubiquitous proteases, such as the proprotein convertase furin, whereas strains causing localized infection are activated by proteases expressed specifically in the respective tissues (3). Until recently it was believed that highly pathogenic avian strains are not transmitted to humans. However, there is increasing evidence that transmission occurs with relatively high frequency. It was first reported in 1996 that an H7N7 virus closely related to an avian strain was isolated from a human case with...
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In the course of an H7N7 outbreak affecting many chicken farms in the Netherlands, 83 human infections were observed. Again most infections were mild, but one case was fatal (6). In 1997, several human infections with a high case fatality rate were observed during an H5N1 outbreak among chickens in Hong Kong (7, 8). In 2003, highly pathogenic H5N1 viruses reemerged in several countries in South East Asia and appear now to be endemic in this region. These viruses continue to be transmitted to humans, again with high case fatality rates (9) (Fig. 2). Although efficient human to human transmission has not been observed yet, the wide spread occurrence of these viruses and their lethality ask for increased efforts to elucidate the molecular correlates of their transmission and virulence.

Highly pathogenic avian influenza viruses cause systemic infection

As a result of the systemic infection caused by the pathogenic H5 and H7 strains in birds, virus can be recovered from many organs. Large hemorrhages distributed all over the body, edema, and cutaneous ischemia are major symptoms of the disease (Fig. 3a). The final stage of the infection is characterized by the emergence of neurological signs, such as hydrophobia and dullness (10, 11). Hemorrhages and edema indicate an affliction of the vascular system and endotheliotropism appears to be a general phenomenon in these infections. Besides endothelia, myocytes and lymphatic tissues were found to be sites of virus replication in some, but not in all studies (12-15). These differences in cell tropism may depend on the developmental stage of the host and on the virus strain used. In contrast, human influenza viruses cause localized infection of human airway epithelium, and recent studies have shown that non-ciliated cells are the primary target cells of these viruses (16).

Figure 1: The structure of influenza A virus. Virus particles contain 8 ribonucleoproteins composed of the genome segments, the nucleocapsid protein NP, and the polymerase proteins PB1, PB2, and PA. The viral envelope is lined at its inner side by the matrix protein M1 and has 3 integral membrane proteins, HA, NA, and M2. NS1 and NS2 are nonstructural proteins. NS2 is also found in virions.

Figure 2: Influenza A outbreaks in man. Influenza A viruses of subtypes H1N1, H2N2, and H3N2 have caused pandemics and have then circled in the human population for the periods indicated. Subtypes H5N1, H9N2, and H7N7 have so far caused only limited outbreaks. The dendrograms show the genetic relationship of the 16 hemagglutinin and 9 neuraminidase subtypes known to date, most of which have so far only been observed in birds (inset).
Determinants of the endotheliotropism of fowl plague virus

Strict endotheliotropism with no virus replication in other cell types was observed, when 11-day-old chick embryos were infected with the A/FPV/Rostock/34 (H7N1) strain of fowl plague virus (FPV) (Fig. 3b). This system is therefore well suited to unravel the factors determining endotheliotropism (17). Genetic analysis showed that the hemagglutinin of FPV is essential for targeted infection of the endothelium. This observation is in line with the concept that, because of its susceptibility to ubiquitous proteolytic activation, FPV HA allows virus entry from the allantoic cavity into the highly vascularized mesenchymal layer of the chorioallantoic membrane and, thus, mediates hematogenic spread of infection. Whereas cleavage activation of HA proved to be essential for targeting the virus to endothelia, it was not responsible for confining the infection to these cells. Furin and the related proprotein convertase PC5/PC6, known to activate FPV HA when originating from mammalian species (18, 19) were identified in all chicken tissues analyzed including endothelial cells. Thus, the lack of spread from infection of endothelia to surrounding tissues could not be attributed to the absence of activating proteases.

In contrast, tissue-specific expression of virus receptors was found to be an important factor in restricting infection to endothelia. With lectin binding assays, α-2,3-linked and α-2,6-linked neuraminic acid, both of which proved to be able to serve as FPV receptors, were detected on epithelial cells and on cells of the reticulo-endothelial system. Similar results have been obtained in a study on the brain microvasculature of the chicken embryo in which another neuraminic acid-binding lectin was shown to selectively bind to the luminal side of the endothelia (20). However, no receptor determinants were found on other cells, such as myocytes, fibroblasts, and hepatocytes. Thus, it appears that cells lacking a measurable amount of neuraminic acid receptors cannot be infected by FPV and are therefore a barrier for the spread of infection. This concept was nicely supported by the observations made on lung tissue. When this organ was infected via the hematogenic route as is the case in the embryo at day 11, the virus was retained in the endothelial cells of the capillary vessels. Since neuraminic acid is present in α-2,6 linkage on these cells, it is clear that this type of neuraminic acid can serve as FPV receptor, although it appears that binding of avian strains is generally determined by the α-2,3 linkage. The alveolar epithelium, although expressing virus receptor in large amounts, are not infected because virus access is prevented by the connective tissue lacking neuraminic acid. On the other hand, when embryos are infected through the airways as can be done by inoculating virus 2 days before hatching into the now almost dry allantoic cavity, virus replication is readily detected in lung epithelia.

It has to be pointed out that expression of neuraminic acid in the chick embryo depends on tissue differentiation (21). This may explain the absence of detectable amounts of neuraminic acid on fibroblasts in situ, whereas cultured fibroblasts readily express receptors as indicated by their ability to allow efficient virus replication. It has also to be assumed that the subendothelial connective tissue is not a very tight barrier in the chori-allantoic membrane where it allows penetration of the virus from the allantoic epithelium into the mesodermal endothelia. In fact, low amounts of virus budding from mesodermal fibroblasts have been observed indicating that these cells may play a role in medi-
The spread of infection through the mesodermal layer is driven by the particularly high virus replication rates in the allantoic epithelium and the presence of neuraminic acid on mesodermal fibroblasts or by some other mechanism remains to be seen.

The polarity of viral budding is another factor contributing to the confinement of infection to endothelial cells. HA was targeted exclusively to the luminal surface of endothelial cells, and virus budding occurred only at this side. The luminal budding polarity of FPV supports therefore the hematogenous spread of the virus and prevents at the same time infection of sub-endothelial cells.

In conclusion, endotheliotropism of FPV in the chick embryo is the result of an interplay of several factors determined by the virus and the host. These include proteolytic activation of HA by ubiquitous proteases, which is responsible for entry of the virus into the vascular system and at least 2 mechanisms contributing to the confinement of the virus to endothelia: the polarity of virus budding at the luminal side of endothelial cells and cell specific differences in the expression of neuraminic acid receptors (Fig. 4). Endotheliotropism without doubt plays an important role in the generalization of FPV infection and in the generation of typical symptoms of the disease, such as hemorrhages and edema. It remains to be seen whether this is only the case in birds, or whether it has also relevance for human infections with highly pathogenic avian viruses. Finally, systemic infection and severe vascular injury are also the central pathogenetic mechanisms of hemorrhagic fevers in primates caused by filoviruses and other agents, and there is evidence that at least some of these viruses replicate also in endothelia (23-25). It will therefore be interesting to see if similar mechanisms as described here for FPV infection play also a role in the pathogenesis of hemorrhagic fevers in other species.

References

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