Cleavability of Hemagglutinin Determines Spread of Avian Influenza Viruses in the Chorioallantoic Membrane of Chicken Embryo

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With 4 Figures

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Summary

The spread of infection in the chorioallantoic membrane (CAM) has been analysed with pathogenic and non-pathogenic avian influenza A viruses. After allantoic inoculation of pathogenic strains, high titers of infectious virus were found in the allantoic fluid, and virus growth could be demonstrated by immunohistology and electron microscopy in the allantoic epithelium, the mesenchyma, and in the chorionic epithelium. By the same route of inoculation, non-pathogenic strains yielded also high titers of infectious virus in the allantoic fluid, but virus replication was restricted to the allantoic epithelium and did not occur in the other cell layers. After chorionic inoculation of pathogenic strains, replication occurred in all layers of the CAM, and infectious virus was released into the allantoic fluid. However, when the chorionic epithelium was infected with a non-pathogenic strain, infection did not spread beyond the site of inoculation. These differences in virus spread are based on differential activation of the hemagglutinin by proteolytic cleavage. The hemagglutinin of pathogenic strains is cleaved in cells of each layer, whereas the hemagglutinin of non-pathogenic strains is cleaved only in the allantoic epithelium. In epithelial cells, virus budding occurred nearly exclusively at the apical side of the cell surface, but this polarization of virus maturation was found with both pathogenic and nonpathogenic strains, indicating that it does not account for the differences in virus spread and, thus, in pathogenicity.

Introduction

Comparative analysis of avian influenza viruses pathogenic and non-pathogenic for chicken revealed striking differences in their range of host cells that produce infectious virus. These differences are based on the susceptibility of the viral hem-
agglutinin (HA) to proteolytic cleavage in an infected cell. Most cell systems investigated are non-permissive for non-pathogenic viruses, i.e. they produce non-infectious virus containing uncleaved HA. In contrast, all cell systems tested are permissive for pathogenic viruses producing infectious virus progeny (2).

It might be assumed that formation of highly infectious virus in a broad range of different host cells is a prerequisite for spread of infection in the organism to exert pathogenic action. This concept which has been derived primarily from cell culture studies in vitro (2, 9, 10, 11, 16) is confirmed in the present paper using chicken chorioallantoic membrane (CAM) as a model organ system. CAM is derived from three germinal cell layers: the chorionic epithelium from the ectoderm, the mesenchymal cells of the connective tissue from the mesoderm, and the allantoic epithelium from the endoderm, which are known to possess different susceptibility to various viruses (12). It could be shown that there is an essential difference in response of chorionic and allantoic epithelial cells to infection with non-pathogenic avian influenza viruses while the pathogenic viruses are produced in both cell layers, similarly. These differences in host range are based on different activation of viral HA by proteolytic cleavage.

Materials and Methods

Virus Strains

The following influenza virus strains were used: A/PR/8/34 (H1N1), A/FPV/Rostock/34 (Hav 1 N 1), A/FPV/Dutch/27 (Hav 1 Neq 1), A/turkey/England/63 (Hav 1 Neq 3), A/parrot/Ulster/73 (Hav 1 N 1), A/turkey/England/647/77 (Hav 1 Neq 1), A/chicken/Germany/49 (Hav 2 Neq 1) (virus N), A/turkey/Ontario/7732/66 (Hav 5 Neq 6) and A/duck/Ukraine/1/63 (Hav 7 Neq 2). The strains underlined were pathogenic for chicken, the others were non-pathogenic (2).

Virus Inoculation of Chick Embryo

Intra-allantoic inoculation was carried out through a hole on one side of 12-day-old embryonated eggs. Chorioallantoic inoculation was done onto the dropped chorioallantoic membrane (CAM) by formation of an artificial air sac (for detail see 12). Inocula contained about 10^4 plaque-forming units (PFU) of the respective virus. Allantoic fluid and CAM were harvested from individual eggs 8, 24, 32 or 48 hours after infection.

CAM Cultures and Radioactive Labelling of Virus Grown either on the Chorionic or the Allantoic Epithelium

For growth of the virus in cells of the allantoic epithelium pieces of CAM adhering to the shell were transferred into a 14 cm Petri dish and embedded in 1.5 per cent agar containing MEM. To grow virus in chorionic epithelium essentially the method described by NAGAI et al. (15) was used. The embedded organ pieces were infected with 50 ml allantoic fluid containing about 10^6 PFU per ml. After incubation at 37°C for 60 minutes the organ cultures were washed twice with PBS and overlayed with MEM containing D-[6-^3H] glucosamine hydrochloride (38 Ci/mmol, Amersham Bucher, Braunschweig) at a concentration of 7 μCi/ml.

After incubation for 20 hours at 37°C the virions were collected by centrifugation at 100,000 × g for 2 hours. The virus pellet was subjected to polyacrylamide gel electrophoresis in the presence of mercaptoethanol and sodium dodecyl sulphate (10).