

H5N1 Virus Attachment to Lower Respiratory Tract

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Highly pathogenic avian influenza virus of the subtype H5N1 may cause infection of the lower respiratory tract (LRT) and severe pneumonia in humans (1). However, the cell types in the LRT to which the virus attaches are unknown for both humans and experimental animals. Although attachment is not the only factor required for virus replication, this information is important both to better understand the pathogenesis of H5N1 influenza and to assess the suitability of animal models. Therefore, we compared the pattern of H5N1 virus attachment to the LRT of humans and four animal species.

Influenza viruses attach to host cells by binding of the hemagglutinin to sialosaccharides on the host cell surface. Human influenza viruses prefer sialic acid (SA)- α -2,6-Gal-terminated saccharides, whereas avian influenza viruses prefer those terminating in SA- α -2,3-Gal (2). The use of lectins that specifically detect α -2,6- and α -2,3-linked sialosaccharides is an indirect measure of influenza virus attachment to host tissues and does not account for other variables that influence the binding avidity, such as type of SA, and glycosylation and sialylation of the hemagglutinin close to the receptor binding site (2). For a more direct method, which was modified from a previously used technique (3), we incubated formalin-fixed, paraffin-embedded tissue sections with formalin-inactivated fluorescein isothiocyanate (FITC)-labeled H5N1 virus (A/Vietnam/1194/04) and detected virus with a peroxidase-labeled rabbit antibody to FITC that was amplified with a tyramide signal amplification system. Tissues comprised histologically normal LRT (including alveolus, bronchiole, and bronchus), as well as trachea from three individuals of each of the following species: human, mouse (C57BL/6), ferret, cynomolgus macaque, and domestic cat (4).

In the human LRT, H5N1 virus attached predominantly to type II pneumocytes, alveolar macrophages, and nonciliated cuboidal epithelial cells in terminal bronchioles. Attachment became progressively rarer toward the trachea (Fig. 1 and table S1). The identity of type II pneumocytes was confirmed by double staining with antibody to human surfactant apoprotein A (fig. S1). These findings fit with the limited pathology data for H5N1 virus infection in humans, which show diffuse alveolar damage (1) and the presence of H5N1 virus antigen in type

II pneumocytes (5). However, they contrast with the idea that avian influenza viruses generally have little affinity for human respiratory tissues (2).

The predilection of H5N1 virus for type II pneumocytes and alveolar macrophages may contribute to the severity of the pulmonary lesion. Because type II pneumocytes are metabolically active and are the most numerous cell type lining the alveoli, targeting of this cell type may lead to abundant virus production. Damage to type II pneumocytes may impair their functions, including re-epithelialization after alveolar dam-

age, ion transport, and surfactant production, and so may inhibit tissue repair. Targeting of alveolar macrophages may be important because of their role in limiting viral replication and in the immune response to viral infection.

The pattern of H5N1 virus attachment to cat LRT and, to a lesser extent, ferret LRT most closely resembled that in human tissues (Fig. 1 and table S1). Based on this criterion, we considered these two species as the most suitable models for H5N1 viral pneumonia in humans. However, other factors also need to be considered, such as the availability of reagents and immunologic similarity. In macaque alveoli, H5N1 virus attached predominantly to type I pneumocytes instead of type II pneumocytes, as in human tissues. In mice, H5N1 virus attachment to cells was most abundant in the trachea and became progressively rarer toward the alveoli, whereas the opposite trend was observed in human tissues. The observed pattern of H5N1 virus attachment to the LRT is consistent with the respective pathology and immunohistochemistry results of experimental H5N1 virus infection in mice (6), ferrets (7), macaques (8), and cats (9).

This study demonstrates the attachment of H5N1 virus to the human LRT in a pattern that corresponds with autopsy findings. It also identifies cat and ferret as the most suitable animal models for human H5N1 viral pneumonia, on the basis of the similarity of viral attachment pattern. This technique also could be applied to further determine H5N1 virus attachment to the upper respiratory tract. Failure to attach to this site may be a limiting factor in human-to-human transmissibility of H5N1 virus.

References and Notes

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10. We thank W. Lim for providing the H5N1 virus isolate; M. den Bakker, H. Sharma, M. Vermeij, and J. van den Brand for providing tissues; and F. van der Panne for technical assistance.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1125548/DC1

Materials and Methods

Figs. S1 and S2

Table S1

References

30 January 2006; accepted 21 March 2006

Published online 23 March 2006;

10.1126/science.1125548

Include this information when citing this paper.

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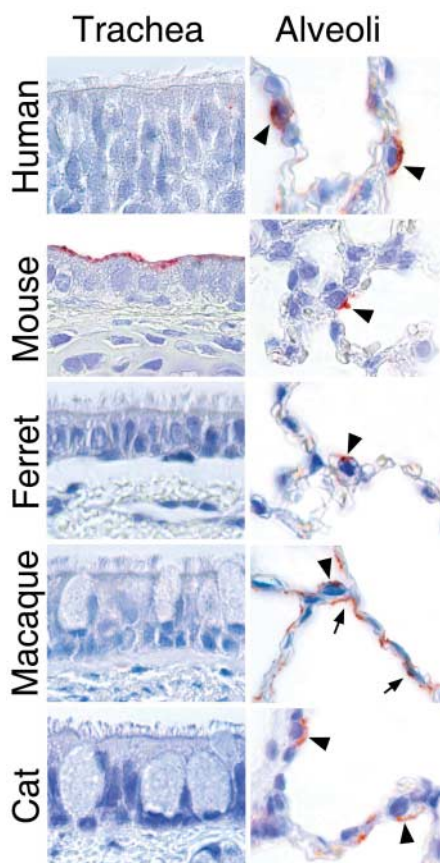


Fig. 1. Attachment of H5N1 virus to respiratory tissues of humans and four animal species. In the trachea, H5N1 virus—visible as red-brown staining—attached only to epithelial cells of mice. In the alveoli, H5N1 virus attached predominantly to type II pneumocytes (arrowheads) in humans and all animal species except the macaque, where attachment was predominantly to type I pneumocytes (arrows).