THE JOURNAL OF INFECTIOUS DISEASES • VOL. 155, NO. 4 • APRIL 1987
© 1987 by The University of Chicago. All rights reserved. 0022-1899/87/5504-0011$01.00

Role of Respiratory Tract Proteases in Infectivity of Influenza A Virus

Charlotte L. Barbey-Morel, Thomas N. Oeltmann, Kathryn M. Edwards, and Peter F. Wright

From the Division of Infectious Diseases, Department of Pediatrics; and the Division of Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee

Proteolytic cleavage of influenza virus hemagglutinin (HA) glycoprotein into subunits designated HA1 and HA2 is required for penetration of virus into the cell. It is generally assumed that this cleavage is an intracellular function of the host cell. Human adenoid fibroblast (HAF) lines, which support the growth of influenza A virus but release virus with an uncleaved HA, provide a model system that has allowed exploration of mechanisms of cleavage in vivo. Exposure of HAF-grown influenza virus to nasal secretions from children with respiratory tract symptoms induced HA cleavage and rendered virus fully infectious. Characterization of this proteolytic enzyme, present in the extracellular environment of the respiratory tract, suggests that it is a serine endopeptidase.

Specific posttranslational proteolysis of a surface glycoprotein is a necessary step in the formation of fully infectious particles for several viruses. Details of the cleavage of the paramyxovirus fusion protein and influenza virus hemagglutinin (HA) were defined by the Choppin laboratory [1–3] with extension of the work by Klenk et al. [4] and Garten et al. [5]. The importance of the cleavability of HA in the pathogenesis of disease due to influenza virus is strongly suggested by studies showing that only virions with cleaved HA have the ability to fuse with membranes and are infectious [6, 7]. The fusion site, which resides buried within the influenza virus HA peptide chain, is only activated by the sequence of proteolytic cleavage of the HA and a conformational change of the HA initiated by lowering the pH to 5 [8, 9]. Entry of influenza virus into cells is envisaged as a multistep process, with attachment of virus followed by adsorptive endocytosis into endosomes, transport into lysosomes, where the pH 5 conformational change occurs with subsequent fusion and transfer of the viral genome to the cytoplasm. A protease provided by the host, of which trypsin is the prototype, cleaves a well-defined site in the HA glycoprotein to form HA1 and HA2. A carboxypeptidase, which is a constituent of the virus, then cleaves arginine from the amino terminus of HA2 [5, 10].

It is generally assumed that these enzymatic cleavages occur in the cell in which the virus is produced. However, in vivo the source of the trypsin-like protease has not been well defined. It remains unclear whether cleavage occurs intracellularly, at the plasma membrane, or extracellularly, after release of virus. In vitro there are three settings in which specific proteolysis of the HA occurs: (*1*) in some continuous cell lines to which exogenous trypsin has been added [11], (*2*) in a limited number of primary cells, of which monkey kidney cells are the best example, and (*3*) in the whole embryonated egg.

To study mechanisms of influenza virus HA cleavage, we have used fibroblast diploid cells grown from human adenoid tissue. These human adenoid fibroblast (HAF) cells can only be infected by a virus with a cleaved HA and in turn release virus with an uncleaved HA. Thus HAFs allow both quantitation of cleaved virus and detection of proteases active against the HA within the human respiratory tract. Using this model, we have exposed uncleaved HAF-grown influenza virus to nasal wash (NW) specimens from children with upper respiratory tract symptoms and have investigated the presence of a trypsin-like protease in nasal secretions.

Materials and Methods

Virus and cells. Influenza A/Bangkok (H3N2) virus that had been isolated from an ill child in January 1981 and grown in embryonated eggs formed the

Received for publication 1 May 1986, and in revised form 15 August 1986.

This work was supported by contract N01-AI-52576 from the National Institutes of Health.

We thank D. Hal Minnigan and Julann Miller for technical assistance. The human adenoidal fibroblast cells were originally characterized by Dr. Gutaro Hashimoto while in Dr. Wright's laboratory.

Please address requests for reprints to Dr. Charlotte L. Barbey-Morel, Division of Infectious Diseases, Childrens Hospital, Washington University, 400 South Kingshighway Boulevard, St. Louis, Missouri 63110.