ZKBS
The Central Committee on Biological Safety

File number 6790-10-71 September 2001

Statement of the ZKBS on the evaluation of the influenza A virus mutant „Delta NS1"

The competent authority of the federal state Hesse asked for the risk assessment and classification of the influenza A virus mutant "Delta NS1" (delNS1) as well as for the classification of genetic engineering operations with this virus.

Introduction

Influenza A viruses belong to the family of *Orthomyxoviridae*. Their genome consists of 8 RNA segments of negative polarity coding for 10 viral proteins (hemagglutinin, HA, neuraminidase, NA, nucleoprotein, NP, matrix proteins: M1 and M2, polymerase proteins: PB1, PB2 and PA, non-structural proteins: NS1 and NS2). The viruses replicate in the respiratory tract of the infected individual. They show a high genetic variability, which is based on their high mutation frequency and the ability for genetic reassortment. The accumulation of point mutations in the two glycoproteins HA and NA leads to antigenic drift. New drift variants are responsible for the occurrence of epidemics and regionally limited outbreaks. In case of simultaneous infection with two virus variants, there may be a re-arrangement between the two sets of 8 genome segments. This phenomenon, the so-called antigenic shift, leads to the emergence of new subtypes (for example, since 1957 H2N2, since 1968 H3N2, since 1977 re-emergence of H1N1).

Influenza viruses are transmitted aerogenically, the contagiousness is high. The diseases range from symptom-poor to severe toxic courses with fatal outcome.

Segment 8 encodes the two non-structural proteins NS1 and NS2, whose nucleotide sequences overlap in 221 bp. At the N-terminus, both polypeptides carry the same 10 amino acids. After this sequence, NS2's mRNA shows an intron deletion of 472 nucleotides, NS1's mRNA is colinear to viral the RNA. After the splicing border at nucleotide 528, the coding sequences for NS1 and NS2 are on different reading frames. NS1 is synthesized shortly after infection and accumulates in the nucleus. NS2 is formed in the late phase of infection and also appears in the nucleus.

During the course of the infection, the NS1 protein, an RNA-binding protein, is involved in various regulatory processes: It inhibits polyadenylation of the host's mRNA, nuclear export and pre-mRNA splicing, influences viral RNA polymerase activity and interacts with host cell proteins. It also inhibits the interferon mediated antiviral immune response via inhibition of protein kinase PKR.

Assessment of the virus

The influenza virus A/PR/8/34 “Delta NS1” belongs to subtype H1N1. The deletion in the NS1 reading frame is set in such a way that the NS2 reading frame is still intact. The gene that counteracts the activity of interferon is thus deleted. As a result, replication is severely restricted and the virus can no longer be reproduced on normal cell cultures (e.g. MDCK). It can be grown in interferon-deficient cells (e.g. Vero). In animal experiments, delNS1 is apathogenic, but the virus is virulent for transgenic mice that lack the interferon alpha receptor (Virology 252, 324 (1998); PNAS 97, 4309 (2000)). By transfection of MDCK cells with the NS1 gene, the defect of the infecting delNS1 can be complemented (PNAS 97, 12289 (2000)).

For the above reasons, the hazard potential of the mutant influenza virus delNS1 can be regarded as lower than that of the wild type virus. However, a downgrading of the risk group is not recommended, as no statements on the attenuation of the mutant can be made at this time. Furthermore, the possibility of accidental double infection of a cell with a circulating wild-type virus and the mutated virus cannot be excluded. At low frequency, the segments may be reassorted and a mixture of viruses may occur, which may contain either the mutant or the wild type segment 8. It is also possible that segments coding for haemagglutinin and neuraminidase, which come from delNS1, are incorporated into coinfecting wild-type viruses. The virus mixture would be assigned to risk group 2.

This classification was adopted unanimously by the ZKBS at its 103rd meeting on 04.09.2001.