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Original article

Amino acid change 335 E to K affects the sialic-acid-binding and neuraminidase activities of Urabe AM9 mumps virus hemagglutinin–neuraminidase glycoprotein

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Abstract

A mutation coding for the amino acid change E_{335} to K is frequently found in the hemagglutinin-neuraminidase (HN) gene of Urabe AM9 mumps viruses isolated during post-vaccination meningitis cases. To identify if this mutation modifies the biological activities of the HN glycoprotein, two variants of Urabe AM9 vaccine differing at amino acid 335 (HN-E_{335} and HN-K_{335}) were isolated and their receptor-binding specificity was determined by means of competence assays. Pre-incubation of the viruses with sialic acids inhibited both syncytia formation in Vero cells and replication in SH-SY5Y cells. Thus, HN-K_{335} showed higher affinity towards sialylα2,6lactose, whereas HN-G_{335} preferred sialylα2,3lactose. These results are relevant because a high expression of sialylα2,6lactose in nerve cells was confirmed by means of *Sambucus nigra* lectin-cytochemistry. In addition, kinetics assays showed that HN-K_{335} and HN-E_{335} also differ in their hydrolysis rate (V_{max} values of 37.5 vs. 3.5 nmol min^{-1} mg^{-1}, respectively). Therefore, HN-K_{335} variant presented a neuraminidase activity level 11-fold higher than that of HN-E_{335} variant. In conclusion, the mutation affects the receptor-binding and neuraminidase activities of Urabe AM9 mumps virus variants.

Keywords: Mumps virus; Hemagglutinin–neuraminidase; Vaccine; Neurovirulence; Point mutation; Sialidase; Sialic acids; Kinetics

1. Introduction

Mumps virus (MuV) infection is an important disease of childhood, sometimes accompanied by complications such as aseptic meningitis, meningoencephalitis, and orchitis, and although much less frequently, sudden deafness [1]. Thus, MuV vaccine is in great demand for protection against mumps virus infection. However, since some vaccines, e.g. Urabe AM9 strain, have been associated with a high rate of post-vaccinal aseptic meningitis, vaccination frequencies of MuV decreased in some countries [2]. The molecular basis of MuV neurovirulence is a problem that must be solved.

MuV is a member of the *Rubulavirus* genus of the *Paramyxoviridae* family and has a single-stranded negative-sense

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RNA genome of 15,384 nucleotides that encodes seven proteins: nucleoprotein, phosphoprotein, matrix, fusion (F), small hydrophobic, hemagglutinin-neuraminidase (HN), and RNA polymerase [1]. Massive vaccination programs have decreased the incidence of MuV infection worldwide [2]; however, mumps epidemics have re-emerged and the incidence is rising in several countries [3]. The main problems associated with MuV vaccination are lack of protection due to vaccine failure (i.e., Rubini strain) and presentation of secondary adverse complications due to the use of relatively virulent vaccine strains; indeed, L-Zagreb, Leningrad-3 and Urabe AM9 strains have been associated with post-vaccinal aseptic meningitis [2,4,5]. Reports indicate that ~1/1000–10,000 recipients of Urabe AM9 mumps virus vaccine developed aseptic meningitis [2,4]. The unacceptably high rate of vaccine-associated meningitis, parotitis and orchitis cases led to removal of Urabe AM9 vaccine from the market in Japan, Canada, and the United Kingdom. However, this vaccine strain is still applied in several countries [5].

Analysis of cDNA sequences of several isolates from vaccine-associated meningitis and parotitis cases demonstrated that Urabe AM9 strain is composed of a mixture of two virus variants that differ at nucleotide 1081 (G/A) in the HN gene. The mutation comprises an amino acid exchange (E/K) at position 335 of the HN glycoprotein; thus, HN-A_{1081} variant codes for Lysin (K_{335}), and HN-G_{1081} codes for glutamic acid (E_{335}) [6]. Further comparisons of the HN gene sequences among several vaccine and wild-type MuV strains led to the identification of an association of HN-K_{335} with neurovirulence [7]. Thus, it was suggested that K_{335} to E mutation in the HN glycoprotein was responsible for attenuation of the wild-type virus. Reversion at this site partially restored virulence of the vaccine [6–8].

Because HN_{335} K/E change avoids recognition of an antigenic site, it was proposed that immune evasion could be part of the advantages of a neurovirulent strain [9,10]. It has also been proposed that this mutation modifies the biological functions of HN glycoprotein, conferring neurotropism for HN-K_{335} virus variant [6,8]; however, there is insufficient experimental support for the participation of HN_{335} change in either virulence or attenuation.

Relevance of the HN-K_{335} mutation for MuV neurovirulence has been questioned because mutations in other viral genes are also associated with the neurovirulent phenotype of mumps viruses [11–14].

We have separated the variants of Urabe AM9 mumps virus vaccine and found that HN-K_{335} virus was efficiently replicated in both human neuroblastoma cells and newborn rat brain, whereas HN-E_{335} virus was replicated at very low titers [15]. Selective enrichment of the HN-K_{335} virus with loss of HN-E_{335} variant after subsequent passage of Urabe AM9 vaccine in human neuroblastoma cells suggested that virus variants differ on their cell attachment capacity [14]. The HN glycoprotein is responsible for mumps virus attachment it binds to sialic acid-containing cell receptors. Its neuraminidase (sialidase) activity releases the sialic acid residues from viral progeny to prevent self-aggregation during budding and activates the F glycoprotein, thereby participating in the internalization of virus particles by fusion of the cell and viral membranes [1]. In this work we experimentally probe that HN-E_{335} and HN-K_{335} variants of Urabe AM9 mumps virus differ in their sialic acid-binding affinity and neuraminidase activity level, and discuss the relevance of these changes in neurovirulence.

2. Material and methods

2.1. Viruses and cells

The HN-K_{335} and HN-E_{335} variants of Urabe AM9 mumps virus vaccine (formerly sold by Smith Kline Beecham) were previously purified by plaque assay. Their purity was verified by both complete sequencing of their HN gene and real-time RT–PCR using an allelic discrimination assay [15]. Viruses were replicated in the green monkey kidney Vero and CV-1P cells and human neuroblastoma SH-SY5Y cells maintained in high glucose Dulbecco's modified minimum essential medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Medium was also supplemented with non-essential amino acids and 8 mM sodium pyruvate for culturing SH-SY5Y cells. All media and culture supplements were purchased from Sigma–Aldrich Chemicals (St. Louis, MO).

2.2. Virus titration

Supernatants of infected Vero cells were obtained at 48 h post-inoculation and centrifuged at 1800 × *g* to separate cellular debris. Viruses in infected cell supernatants were quantified by standard plaque assay in CV-1P cells overlaid with soft agar. Results were reported as plaque-forming units (PFU), and in all the following experiments cells were infected with 0.2 PFU/cell of each virus variant.

2.3. Enzyme treatment of susceptible cells

SH-SY5Y cell monolayers were cultured on 24-well plates, at 24 h after seeding cells were treated with serial dilutions of *C. perfringens* neuraminidase for 30 min at 37 °C. Cells were then washed twice with PBS and inoculated with each virus variant [16]. Untreated SH-SY5Y cells were also inoculated with both viruses and used as controls. Viral titers in recovered supernatants were determined by PFU in CV-1P cells at 48 h post-inoculation [15].

2.4. Oligosaccharide competition assays

For competitive antiviral assays, each virus variant was mixed with 25–400 μM of 5-*N*-acetylneuraminic acid (Neu5Ac), sialylα2,3lactose, sialylα2,6lactose, lactose or D-galactose or 25–200 μg/ml of colominic acid (α2,8-linked polysialic acid) and incubated 1 h at 4 °C. Vero and SH-SY5Y cells (5×10^4/well) seeded in 24-well culture plates were inoculated with the mixtures of virus and oligosaccharides