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NEURAMINIDASE ACTIVITY AND ANTIGENICITY OF THE EGG ADAPTED STRAINS OF MUMPS VIRUS

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Summary. — Two freshly isolated mumps virus strains were adapted to embryonated eggs. The activity of their neuraminidase (NA) and their antigenicity were examined in the 5th, 10th and 15th passages. The NA was characterized by estimation of the Michaelis constast, while the antigenicity of virus strains was assessed by induction of antibodies as detected in complement fixation (CF) tests. The K_M value for strain Berlin 9/76 NA in its 15th passage was 5.65 mmol/l and exceeded 4.2 times that of strain Berlin 1/76 (K_M = 1.31 mmol/l). The K_M values of both strains increased in the course of passaging by 55 or 260%, respectively. The antigenicity of both egg passaged strains remained unchanged. Under the same conditions, the Jeryl Lynn vaccine strain revealed a seroconversion rate of 44%; the K_M value of the NA from this strain was closely similar to that of Berlin 1/76 virus in the 15th passage (= 1.34 mmol/l).

Key words: mumps virus, neuraminidase, Michaelis constant, egg adaptation, antigenicity

Introduction

As described by Henle and Deinhard (1955), passaging of mumps virus strains in chick embryos leads to adaptation and under suitable conditions to attenuation of the virus. A safe and standard attenuation procedure is the prerequisite of live vaccine production. In the case of mumps virus, however, a correct estimation of the degree of attenuation is difficult. Our investigations aimed to correlate the Michaelis constant (K_M) of viral NA to the degree of adaptation or attenuation of mumps virus strains utilizing K_M as markers. The changes occurring during passaging in the amnion cavity at low temperatures were compared to the antigenic variations of virions.

Materials and Methods

Viruses. The live (attenuated) mups virus vaccine strain Jeryl Lynn (Mumpsvax, Behringwerke AG, Marburg/Lahn, F.R.G.) was passaged 3 times through embryonated eggs amnion cavity. The laboratory passaged strain Enders has undergone 55 passages in the allantois. Strains Berlin 1/76 and 9/76 were isolated in 1976 from two sick children showing typical clinical symptoms by inoculation of saliva and pharyngeal swabs into the amnion cavity and subsequently passaged 15 times. Their biochemical and biological characteristics were determined in the 5th, 10th and 15th passages.

Table 1. Michaelis constants of the neuraminidase of different mumps virus strains

Virus strain	Number of egg passages				
	5	10	15	18	55
Berlin 1/76	0.86*	1.39	1.31	—	—
Berlin 9/76	1.23	3.14	5.65	—	—
Jeryl Lynn	—	—	—	1.34**	—
Enders	—	—	—	—	1.77

* in mmol/l

** 3 passages in the amnion cavity in addition to 15 passages in chick embryo fibroblasts.

Immunization. Male guinea pigs weighing 200—250 g, free of antibodies to mumps virus, were used for immunization. Each animal received intranasally 1000 ID_{50} per dose. For each virus strain 20 guinea pigs were employed. Exsanguination was performed 21 days after administration, of antigen. Sera were examined by complement fixation (CF) performed in microplates (Bonin. 1973) with standard parotitis virus antigen (Mumpsvirus Testantigen IAV Berlin, G.D.R.) CF antibody titres ≥ 16 were regarded as positive. Sera revealing the highest titres were investigated by NA inhibition test according to Aymard-Henry et al. (1973).

Neuraminidase. The enzyme activity was determined in a modified test differing from that recommended by the WHO for influenza virus (Aymard-Henry *et al.*, 1973) as follows:

— the substrate fetuin was replaced by cheaper ovomucoid (100 mg/ml)
— the incubation period at 37 °C was cut down to 4 hr
— measurements of N-acetylneuramic acid (NANA) released from the substrate were performed by means of a modified thiobarbituric acid procedure (Seidel, 1978, personal communication Nhung and Ha, 1979) using tri-ethyleneglycol as chromophor solvent.

NA activity as determined according to WHO procedure reveals higher Michaelis constants than found by Seidel's test (1978), because in the former test, in addition to NANA, also other saccharides are converted into chromophores (Culling *et al.*, 1977). The sequence of K_M values as detected by the two different NANA estimating methods remained the same.

Results and Discussion

Table 1 shows the Michaelis constants (K_M) of the NA from mumps virus strains Berlin 1/76 and Berlin 9/76 on one hand and from mumps vaccine strain Jeryl Lynn and laboratory strain Enders on the other hand, as related to the number of passages. Comparison of data obtained revealed enhancement of the constant by 50—60% in the course of passaging of the Berlin 1/76 strain. The K_M values for the strain Berlin 9/76 NA were higher in general. The increase observed during passaging was considerably more prominent, namely 155 and 360%. In the 15th egg passage the K_M of this strain was 4.2 higher than that of the Berlin 1/76 strain, while in the lowest passage examined the difference took 43% only. When these K_M values are compared to that of the Jeryl Lynn strain NA, the low levels of the latter are similar to strain Berlin 1/76 in the 10th and 15th passages. The K_M value of the laboratory passaged Enders strain is higher than those of the Berlin 1/76 strain after 10 and 15 passages, but is still lower than those of Berlin 9/76 after 10 and 15 passages. The reason of the lower NA K_M values of both Jeryl Lynn and Enders strains reported here in comparison to our previous findings (Klamm, 1980) has been explained in Materials and Methods. The

Table 2. Rates of seroconversion and geometric mean titres of antibodies induced by mumps virus strains in guinea pigs

Virus strain	Number of egg passages			
	5	10	15	18
Berlin 1/76	100 (74)*	82 (205)	100 (116)	—
Berlin 9/76	100 (103)	100 (92)	100 (174)	—
Jeryl Lynn*	—	—	—	44 (39)

* Mean CF antibody titres in brackets.
** Legend as in Table 1.

process of egg adaptation is reflected in the K_M values of NA by decreased affinity of the enzyme for substrate, i. e. the efficiency of enzyme activity becomes impaired. The biological significance of altered biochemical activity of NA in association with the degree of attenuation is, however, still unclear.

The Michaelis constant for vaccine strain Jeryl Lynn NA does not represent an extreme value in comparison to K_M values of other virus strains or variants. Therefore, this value can not be regarded as limiting for NA of an optimal attenuated mumps virus strain. By estimating the K_M of NA in the course of passaging (adaptation) of a certain virus strain it became possible to assess alterations of the virus. This notion is supported by results of McNulty et al. (1975) who showed that NA of a virulent NDV strain had considerably higher specific activities as compared to the low virulent ones.

Results of antigenicity testing in guinea pigs were expressed as the rates of seroconversion and as geometric means of CF antibody titres (Table 2). The two freshly isolated mumps virus strains at different passage levels induced a nearly 100 per cent seroconversion. After 15 egg passages the antigenicity of mumps virus strains Berlin 1/76 and Berlin 9/76 has not decreased. In contrast, the seroconversion achieved with the Jeryl Lynn strain was 44% only. It should be considered that the sera were examined in CF reaction. The more sensitive neutralization test could have changed the balance more in favour of the Jeryl Lynn strain. In addition, the mumps virus strains grown in chick embryo cells (i. e. the Jeryl Lynn strain) are known to be less antigenic than those grown in embryonated eggs (Schramek, 1969). All this should be taken into account when evaluating the serologic results.

The determination of neuraminidase inhibiting antibodies in guinea pig sera with the method of Aymard-Henry (1973) was unsuccessful probably due to the low sensitivity of the test. This procedure, however, proved suitable when haperimmune guinea pig serum was used (results not shown). No parallel between biochemical and antigenic alterations of the NA could be demonstrated, because of the failure of NA inhibition test. Such a parallel might not exist either, as the antigen determinant(s) and the active site of the enzyme molecule need not coincide.

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