Acta virol. **34** : 85—89, 1990

AN ATTEMPT TO ANALYSE THE FUNCTIONAL DIFFERENCE BETWEEN VARIOUS MUMPS VIRUS STRAINS

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Received April 11, 1988

Summary. — Enzymatic activity of N-acetylneuraminidase of ten various strains of mumps virus was compared. From the viewpoint of their biological properties, these strains could be classified as laboratory, neurovirulent, and vaccinal (attenuated) ones. The enzymatic activity was evaluated by Scatchard's plot which enables to interpret it according to polarity of the cooperativity of enzymes' binding sites. Laboratory strains of the mumps virus demonstrated an independent type of cooperativity, while vaccinal (attenuated) ones showed a positive type of cooperation.

Key words: *mumps virus*; *N-acetylneuraminidase*; *enzyme kinetics*; *Scatchard's plot*; *cooperativity of enzymatic activity*

Mumps virus (MuV, Family Paramyxoviridae, Genus Paramyxovirus) is an enveloped virus the membrane of which containe antigenic spikes of two species of glycoproteins namely the HN (haemagglutinin-neuraminidase) and the F glycoprotein (fusion factor) (Örvell, 1978). Both glycoproteins form antigenic structures which are the important factors of virulence at contact and interaction with host cell. The different biological properties of various mumps virus strains are given by qualitative and quantitative differences in functional and structural properties of these virus surface antigens. We deal only with the analysis of virus-specific enzymatic activity of N-acetylneuraminidase (NA, E.C. 3.2.1.18.) the HN glycoprotein. Some previous papers have been devoted to the study of MuV NA activity. Merz and Wolinsky (1981) determined the Km values (Michaelis-Menten's constant) and Vmax values (maximal reaction rate) for NA activity of some model strains. Similar study with another group of MuV strains was performed by Klamm (1980) and Klamm and Pollex (1982) who, in addition, desribed the changes of Km during the propagation of the mumps virus strain. In another study they paid attention to the question of variability of NA of various MuV strains in their substrate demands according to the glycosidic bound type of the substrates tested (Klamm and Pollex, 1984). Inhibition tests *in vivo* with sets of monoclonal antibodies confirmed the

Table 1. Survey and characterization of MuV strains

No.	Name	Strain	Cell substrate (No. of passages)
1	Enders-KE	Laboratory	CE (160)[a]
2	Enders-Vero	Laboratory	Vero (11)
3	MP-45	Neurovirulent[b]	Vero (2)
4	MP-45/CNS-8	Neurovirulent	CNS-hamster (8), Vero (2)
5	Jeryl Lynn B	Vaccinal (MUMPSVAX, U.S.A.)	Vero (2)
6	Jeryl Lynn B	Vaccinal	DK (6)[c]
7	Jeryl Lynn B	Vaccinal (MUMPSVAX, U.S.A.)	DK (6), Vero (1)
8	Leningrad 3/—1	Vaccinal (U.S.S.R.)	JQEF (19)[d], Vero (2)
9	Leningrad 3/—2	Neurovirulent (for monkeys)	JQEF (7), Vero (2)
10	Leningrad 3/—3	Vaccinal (hyperattenuated)	JQEF (cca 30), Vero (2)

[a] Chick embryo
[b] Virus was isolated from patient
[c] Primary culture of dog kidney cells
[d] Primary culture of Japan quail embryo cells

assumption of the central role of functional activities of HN glycoprotein in the pathogenesis of MuV infection (Löve *et al.*, 1985).

The tested MuV strains are listed and characterized in Table 1. Enders strains were from the collection of SEVAC Prague, MP-45 strains were isolated from cerebrospinal fluid of a patient (Regional Station of Hygiene, Ostrava, Czechoslovakia), Leningrad strains were obtained from Dr. N. N. Gordienko (Research Institute for Viral Preparates, Moscow, U.S.S.R.),

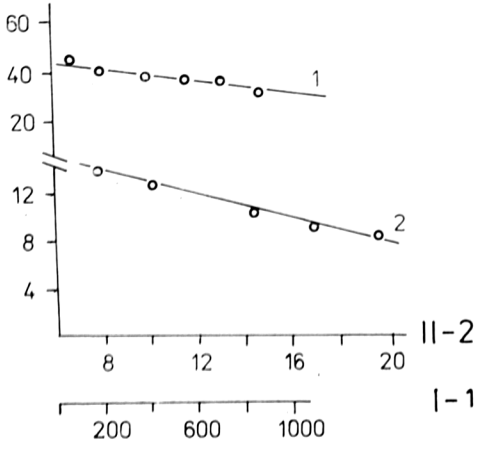

Fig. 1.
The independent type of cooperativity of N-acetylneuraminidase binding sites Abscissae: I-1 and II-2: $v \times 10^8$ (mol $\times$ $\times$ $mg_p^{-1} \times hr^{-1}$); ordinate: $\frac{V}{S} \times 10^2$
1 — Enders-KE; 2 — Enders-Vero

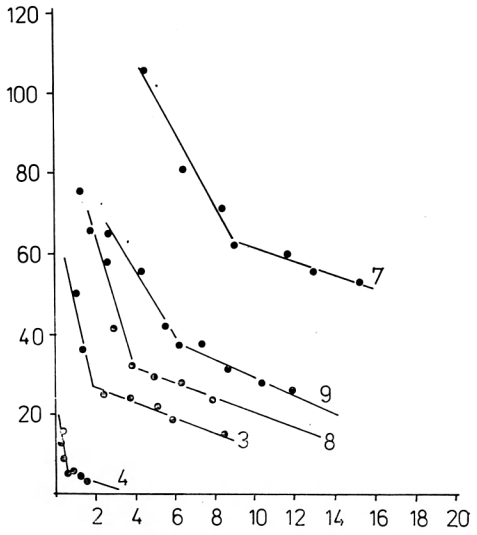

Fig. 2.

The negative type of cooperativity of N-acetylneuraminidase binding sites
Abscissa: $v \times 10^3$ (mol $\times$ $mg_p^{-1} \times hr^{-1}$);
ordinate: $\frac{V}{S} \times 10^2$
3; 4; 7; 8 — mumps virus strains, see Tab. 1

Jeryl Lynn B strains came from commercial vaccines (MUMPSVAX, U.S.A. and SEVAC, Czechoslovakia). Individual strains were propagated in cells shown in Table 1; after clarification they were concentrated by ultracentrifugation at 45 000 g, washed in PBS, sedimented at 100 000 g and resuspended into the McIlvain's buffer at optimal pH for the NA of MuV strain. Another purification by ultracentrifugation over the linear density gradient of succrose 15—55% (w/w) was performed with the Enders-Ke strain. The enzymatic activity of viral NA was measured by a standard technique according to Aymard-Henry *et al.* (1973). Fetuin (SEVAC, Czechoslovakia) was used as a substrate under the pH-optimum conditions for enzymatic activity of each strain. The enzymatic reaction rate of NA related to the protein weight of the tested strain was expressed in mol . mg^{-1} . hr^{-1}. The kinetics of reactions was expressed by Scatchard's plot (Scatchard, 1949; Kotyk and Horak, 1977). The protein concentration in tested preparates of MuV was determined according to Lowry *et al.* (1951). As a standard the bovine serum albumin was used (fraction V, Armour, London).

The velocity kinetics of the enzymatic reaction of viral DNA were expressed by Scatchard plots (Figs 1, 2, and 3). Fig. 1 shows the Enders strains, which reveal an independent type of cooperation of the binding sites. Fig. 2 depicts all strains with negative cooperation, while the strains with a positive type of cooperation of their binding sites are shown on Fig. 3.

Interpretation of the most accessible kinetic parameters (K_m, V_{Max}) of of enzymatic activity of NA MuV from the point of view of interstrain differences is very complicated and confusing. The biological role of this virus-specific enzymatic activity keeps unclear (Smith and Hightower, 1983). The level of virulence of MuV strains, or degree of their attenuation during

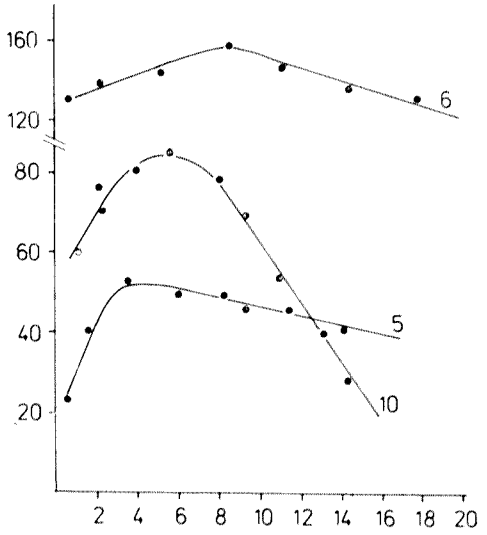

Fig. 3.

The positive type of cooperativity of N-acetylneuraminidase binding sites
Abscissa: $v \times 10^8$ (mol × mg_p^{-1} × hr^{-1});
ordinate: $\frac{V}{S} \times 10^2$
5; 6; 9 — mumps virus strain, see Tab. 1

preparation of live vaccinal strains is a result of complex function-structural effect of all antigenic structures of MuV virions. Some authors estimate from the high K_m value due to the low NA activity the neuropathogenic effect of the strains; however, such data are obtained with a model substrate (Merz and and Wolinsky, 1981). They reflect only the phasis of budding of virions because virions from such a strain have a real opportunity to remain comparatively longer in close contact with the membrane of host cell. Though, this idea doesn't take into account the importance of NA glycoprotein HN in the adsorptive and receptive phases, eventually in the intracellular stage of infection. Klamm and Pollex (1982) consider the connection between the NA MuV activity and degree of attenuation of MuV strains for very unclear. The functional NA activity is one of the complex of three viral specific activities, which is topologically related to the structure of integral glycoprotein HN in the membrane of the MuV virion. Consequently, it is an allosteric enzymatic system activity. That is why we selected the plot of kinetic parameters of NA according to Scatchard (Kotyk and Horak, 1977) for the analysis of the NA MuV activity and its correlation with the biological character of corresponding strains of MuV. Character of obtained graphic relationships enabled us to distinguish the studied MuV strains according to the polarity of the cooperativity of binding sites of the NA enzymatic activity dividing them into three groups, generally in harmony with dividing the individual MuV strains according to their biological properties.

The first group represented by the laboratory strains Enders showed the independent type of cooperativity (Fig. 1). The second one, represented by neuropathogenic strains (including the L3/-2 strain pathogenic for monkeys)

showed the negative type of cooperativity (Fig. 2). To this group belong also the vaccinal strains Jeryl Lynn B (DK 6) and L3/-1 which underwent one to two last passages on Vero cells. During these two passages they either had changed the character of the functional dependence of NA activity or heterogenic binding sites emerged in enzymatic NA systems. The third separated group are strains with a positive type of cooperativity. These are the vaccinal attenuated strains of American live vaccine MUMPS-VAX-Jeryl Lynn B and Jeryl Lynn B (DK 6) and also the hyperattenuated strain L3/-3 derived from the Russian live vaccine L3/-1 (Fig. 3). Although we did not succeed in explaining the mechanism of revealed correlation, we try here to evaluate exactly the properties of various MuV strains what could enable us to measure the degree of attenuation of potential vaccinal strains and the stability of MuV vaccinal strains, respectively. In spite of the fact that we made a point that the whole problem cannot be reduced to study only one functional activity, it has been demonstrated that this way of interpretation of enzymatic activity of NA could complement and detail the classic biological tests for the control of the live mumps vaccine.

Acknowledgement. Authors are very grateful to the Professor Dr. Dimitrij Slonim for valid advice and kind reviewing of the manuscript. We wish to thank also to Mrs. Jaroslava Hauserová for the kind help with the manuscript preparation.

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