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COMPARATIVE STUDY ON THE NEUROVIRULENCE OF DIFFERENT VACCINE STRAINS OF PAROTITIS VIRUS IN MONKEYS

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Summary.— The neurovirulence of several mumps virus vaccine strains used for preparation of vaccines in various countries was compared in 44 green monkeys and in 36 *Macaca mulatta* monkeys. The residual neurovirulence of the attenuated mumps virus strains was demonstrated by morphological examination revealing the specific localization and character of brain lesions after intracerebral (i.c.) administration. We found that the affinity of mumps virus to ependymal cells determines the development of serous meningitis after vaccination with the mumps virus vaccine.

Key words: neurovirulence; mumps virus; attenuation; vaccine

Introduction

At present, morphological control of the neurovirulence of mumps virus vaccine is one of the principal methods for checking its safety. We have demonstrated earlier qualitative dissimilarities in the character of CNS changes in monkeys after infection with mumps virus strains of different virulence (Yuzepchuk *et al.*, 1975). These dissimilarities have been proposed as morphological criteria for estimation of the attenuation. On the other hand, Buynak and Hilleman (1966) using the same technique did not observe any differences in the characteristics of the CNS lesions in monkeys infected with strains of different virulence.

In the framework of a wide prophylactic vaccination in the U.S.S.R. and some other countries this problem became of practical importance, both in evaluation of neurovirulence of the vaccine strain and in analysis of the cause of postvaccination neurological syndromes. Therefore, a comparative study in monkeys of the strains used for vaccine preparation in different countries has been carried out with the goal to determine the morphological parameters of the vaccine strain and to standardize the method of its preparation control.

Materials and Methods

Animals. Monkeys of two species were used: green monkeys and *Macaca mulatta*, seronegative

with respect to mumps virus. The animals were infected in both hypothalami under hexenal anaesthesia and were housed in separate cages. Culture fluid of noninfected cultures was injected as control. The experiments were carried out for 3 weeks.

Viruses. Following virus strains and variants, respectively, have been used: 1. The vaccinal strain used for preparation of live mumps vaccine in the U.S.S.R. — L-3(22)* and 2. an insufficiently attenuated variant of the same strain — L-3(7). More detailed data on the history of attenuation were described elsewhere (Yuzepchuk *et al.*, 1975) 3. Strain Sofia-6 used for manufacturing of the vaccine in Bulgaria. 4. Original vaccine of the strain Jeryl Lynn manufactured by Boehring, batch No. A 14004. 5. Freshly isolated strain of mumps virus prepared in the Institute of Applied Virology (Berlin, G.D.R.). Each monkey was infected with 25,000 to 30,000 HAU_{50} of the virus-containing fluid. The brain was fixed in 10% formalin. Paraffin sections of 5—7 μm thick were stained according to Nissl and with haematoxylin-eosine.

Results

In the first experimental series the pathological changes in the CNS of monkeys after injection of the freshly isolated wild strain Berlin and of the attenuated strain L-3(22) were compared. Monkeys of the same species (green monkeys) were used in this experiment; 8 monkeys were infected with each of tested viruses. No neurological symptoms were observed during clinical examination of either group. The gross somatic changes were the following: rise in temperature, enlargement of parotid glands and food rejection in monkeys infected with the virulent strain. However, these changes were seen by only 2—3 days post infection (p.i.) and disappeared on days 4—6 p.i.

In the brain of monkeys infected with the freshly isolated strain diffuse infiltration of meninges, and choroid plexi were detected. In the meninges on the basal and external surfaces of large hemispheres accumulations of lymphocytes were found. The ependyma of the lateral, as well as of IIIrd and the IVth brain ventricles underwent desquamation resulting in exposure of basal membrane; in some areas nodular proliferation of the epithelium was observed (Fig. 1). Under the ependyma separation of the astrocytic barrier fibres and oedema were seen. Choroid plexi were widened, oedematic and markedly engorged. Around the veins of distal regions of the plexi tight mononuclear cuffs were seen. Changes were found predominantly in the anterior and posterior horns of the lateral ventricles, as well as the IVth brain ventricle. Around the needle truck small-focal destruction of neurons was observed. Neuronal destruction also occurred in the hippocampal cortex, transparent septum and praepyriform region of the cerebral cortex (Fig. 2).

No changes were found in the meninges of monkeys infected with the L-3(22)strain. No destruction of ependymal cells was observed, but nodular proliferation of the ependyma has occurred. Choroid plexi were not enlarged. Lymphohistiocytic perivascular infiltration was seen in the plexi of lateral brain ventricles only (Fig. 3). Outside the damaged area no neuronal destruction was found (Table 1).

The next experimental series dealt with the changes in the brain on the infection of monkeys by different vaccinal strains, as compared to low-atten-

* Given in brackets are the numbers of passages in cell culture. Vaccine batch No. 258 manufactured by the Moscow Viral Preparations Manufacturing House (1981) was used as vaccine strain.

Table 1. Morphologic comparison of pathogenicity of wild and vaccine strains of mumps virus

Strain	Virus dose (HAU_{50})	Number of monkeys with pathomorphological changes			
		neurons	ependyma	choroid plexi	meninges
Isolate (Berlin)	30,000	8	8	8	6
L-3(22)	30,000	0	8	8	0

uated strain L-3(7) of mumps virus (Table 2). Clinical examination of all the monkeys did not show any pathological changes. Microscopic examination of the brain lateral ventricles of monkeys infected with the L-3(22) strain revealed signs of chorioplexitis and ependymitis. No destructive changes of parenchymal elements were seen in the mentioned areas, only infiltration and proliferative changes were present. These changes , though differently marked, were observed in all monkeys. However, necrobiosis of neurons was found only in the area of injection truck.

After infection with the L-3(7) strain changes were found in the neurons of the paleocortex and archicortex, as well as in the ependyma and choroid plexi of all the ventricles in monkeys of both species. Loss of neurons was observed only in the hippocampus, transparent septum and in the peritraumatic zone (Fig. 4). Sixteen monkeys were infected with the strain Sofia -6. Green monkeys appeared more susceptible. In addition to the changes in choroid plexi and ependyma, focal loss of single neurons was found in trapezoid body and periventricular zone around the anterior horn in 3 monkeys (Fig. 5). The changes in choroid plexi and ependyma were limited and did not affect all brain ventricles. They were predominantly found in the lateral ventricles, in 2 *Macaca mulatta* and in 1 green monkey — in the IVth brain ventricle. No necrotic changes in the ependyma and choroid plexi were observed.

Table 2. Comparison of pathogenicity of different strains of mumps virus

Strain	No. of monkeys		Virus dose (HAU_{50})	Pathomorphological changes					
	Macaca mulatta	green monkeys		neurons		ependyma		choroid plexi	
				M	G	M	G	M	G
L-3(222)	10	6	30,000	0/10	0/6	8/10	6/6	9/10	5/10
L-3(7)	8	10	30,000	0/8	0/10	8/8	10/10	8/8	10/10
Sofia-6	10	6	30,000	3/10	1/6	8/10	6/6	6/10	5/6
Jeryl-Lynn	8	6	30,000	2/8	3/6	8/8	6/6	8/8	5/6

Notice: Numerator — number of monkeys with observed changes;
denominator — number of animals.
M — Macaca mulatta, G — green monkeys.

The administration of strain Jeryl Lynn to *Macaca mulatta* and green monkeys was followed by brain changes of different degree. Green monkeys appeared more susceptible to this strain. In *Macaca mulatta* the inoculation of Jeryl Lynn strain was followed by changes not only in the ependyma and brain choroid plexi, but also in the periventricular formations. In all the monkeys lymphocytic infiltration was seen around the vessels in the zone of lateral ventricle horns, and in 2 monkeys — around the IVth ventricle (Fig. 6). Ependymal destruction was focal, scarce perivascular lymphocytic infiltration was found in the choroid plexi.

The administration of strain Jeryl Lynn to green monkeys was followed by marked oedema in the ventricular walls with swelling ependyma and epithelium of choroid plexi. In walls of all brain ventricles, but mostly of the lateral ones, the fibres were separated and soaked with serous exudate: fibrinoid degeneration was found in the vessels (Fig. 7). In the same area different dystrophic neuronal changes were present. In the vicinity of the needle truck, destruction of neurons and lymphocytic „cuffs" around vessels were apparent (Fig. 8). In the meninges loosening of fibres was seen and the pial spaces were filled with serous exudate. In general, the observed changes in this monkey species were upmost similar to allergic inflammation.

Thus, the neurovirulence test with different mumps virus vaccine strains in monkeys detected a residual neurovirulence in all of strains tested, although their neurotropic properties had been lost during attenuation.

Discussion

Comparative studies of CNS pathology in different viral infections proved that the changes in monkeys are the most similar to man (Bodian, 1956; Nathanson *et al.*, 1968; Robinson, 1957; Khesin *et al.*, 1961; Rozina, 1972). Although the nervous system is fairly often (30% and higher) involved, in some epidemics of parotitis (Tsuker, 1972; Bondarenko and Freidkov, 1981; Oklitz 1981) usually there are no lethal cases, so that the CNS lesions cannot be studied. Experimental meningoencephalitis was elicited in monkeys (Gordon. 1927) providing that freshly isolated virus from a patient's saliva was administered by i.c. route.

Studying the CNS changes in monkeys infected with neurovirulent and attenuated strains of mumps virus we came to conclusion that the degree of attenuation could be different. The U.S. National Standards for production of mumps vaccine requires a neurovirulence control: the absence of pathohistological changes in the CNS of infected monkeys indicating the absence of a neurotropic agent is a prerequisite. In our opinion, such a definition is not precise enough as it does not specify the criterions of the test. Our previous experiments in monkeys with mumps virus strains of different virulence demonstrated that it is essentially possible to estimate the degree of attenuation of the strain according to morphological characteristics of lesions induced in the CNS (Yuzepchuk *et al.*, 1975; Rozina *et al.*, 1984).

After i.c. administration of attenuated strains changes were observed in the

brain ventricular structures only, while virulent strains caused also neuronal damage.

It follows from our findings that loss of neurons was observed after administration of the low attenuated strain L-3(7) and of the freshly isolated strain „Berlin". The comparison of neurovirulent properties of different vaccine strains allowed to evaluate the criteria for appropriate manufacture control. It can be seen from these results that all vaccine strains caused changes only in the ependyma and choroid plexi of brain ventricles. In general, this was characterized as a limited ependymitis and chorioplexitis, predominantly affecting the structures adjacent to lateral brain ventricles. Another specific feature of the pathological process caused by the vaccine strains was: the absence or insignificant alteration of neurons in brain structures involved in the inflammation manifested by its infiltration and proliferation components.

Thus, the localization of the process, predominantly inflammatory character of the changes in the ependymal lining and vascular plexi of brain ventricles as well as the absence of neuronal necrobiosis were the main criteria for safety of the mumps virus for vaccine preparation. Our experimental findings shed light on the probable pathogenesis of serous meningitis, developing in epidemic parotitis patients. We believe, that the damage to ependyma and brain choroid plexi observed in the experiment, are responsible for cerebrospinal fluid disturbances.

The observed virus-specific changes of the brain ventricular structures functionally participating in production and absorption of cerebrospinal fluid in monkeys infected with mumps virus vaccinal strains disclose the probable mechanism of postvaccinal serous meningitis in vaccinated children.

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Explanation of Figures (Plates XXXVI—XXXIX):

Fig. 1. Anterior horn of lateral ventricle. Oedema, loosening of the astrocytic barrier, perivascular infiltration. The ependyma is peeled off in some places, the basal membrane being exposed. Magn. 300×. Here, in Figs 2—4 and 6—8 haematoxylin-eosine stain.

Fig. 2. Transparent septum of the brain. Microfoci of necrosis in the parenchyma, perivascular infiltrates, desquamation of ependyma. Magn. 270×.

Fig. 3. Choroid plexus of the brain lateral ventricle. Venous hyperaemia, mononuclear infiltration of the stroma. Magn. 360×.

Fig. 4. Multiple foceal necroses in the perivascular zone of the lateral ventricle. Perivascular infiltrates below the ependyma, desquamation of ependymal cells. Magn. 300×.

Fig. 5. Microfoci of neuronal destruction in the trapezoid body in pons varoli. Nissl stain, magn. 900×.

Fig. 6. Round-cellular perivascular infiltration in the periventricular zone. Ependyma is intact. Astrocytic barrier is loosened. Magn. 450×.

Fig. 7. Widerspread oedema and infiltration of the lateral ventricular wall, Ependyma is desquamated. In the centre fibrinoid degeneration of vessels. Magn. 400×.

Fig. 8. Focus of neuron destruction in the prerivascular zone of the ependyma. Oedema, the barrieris loosened. Magn. 600×.