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STUDY OF MUMPS VIRUS INVASIVENESS IN MONKEYS

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Summary. — Invasiveness of mumps virus strains, differing in the degree of attenuation was studied in green and *Macacca mulatta* monkeys infected either into salivary glands or intramuscularly. Peripheral infection with all strains under study resulted in their penetration into CNS irrespective of the attenuation degree. Specific morphological changes were observed in the brain of monkeys as confirmed by detection of virus antigen using fluorescent antibodies (FA) staining. Morphological changes and time of virus antigen detection in the brain differed depending on the neurovirulence of the strains based on different degree of their attenuation.

Key words: *neurovirulence*; *invasiveness*; *mumps virus*; *antigen persistence*

Introduction

Among neuroinfections in childhood lesions of CNS due to mumps virus are of considerable importance. Frequency of CNS involvement in the pathology of mumps virus (MV) infection ranges from 8 to 15 % (Bondarenko and Freidkov, 1971; Oklits, 1981). Experimentally, meningoencephalitis of MV aetiology develops in monkeys inoculated intracerebrally (i.e.) with wild MV strains (Gordon, 1927; London and Kent, 1979). Vaccination with live mumps vaccine strain "Jeryl Lynn" was accompanied by the appearance in vaccinees of mumps-specific symptoms including meningeal reactions (Hayden *et al.*, 1978; Quast *et al.*, 1979). At present, however, there is no direct evidence for the aetiological role of the vaccinal strains in development of these symptoms, but experimental study on invasive qualities of the attenuated mumps virus strains was not carried out.

The aim of the present research was to investigate the invasiveness of MV strains, differing in the degree of attenuation using green and *Macacca mulatta* monkeys infected intramuscularly (i.m.) and into the salivary glands.

Materials and Methods

Mumps virus strains. Two variants of the Leningrad-3 (L-3) strain were used: 1) the vaccinal variant used for production of live mumps vaccine in the U.S.S.R., designated in this report as L-3 (22). Isolation, attenuation and characterization of this strain were described previously (Nasibov and Smorodincev, 1969); 2) insufficiently attenuated variant of the same strain designated L-3 (7), biological characteristics of which were described by Vardanjan (1973) and

Yuzepchuk *et al.* (1975). The original S-40 strain was identified in collaboration with Dr. Odiseev from Research Institute of Contagious and Parasitic Diseases in Sofia in 1976 (Yuzepchuk *et al.*, 1975). The vaccine strain "Jeryl Lynn" was purchased from Behringwerke (series A-14004).

Animal experiments. Green monkeys and *Macacca mulatta* monkeys weighing 2—3 kg were lacking virus-neutralizing antibodies (NA) to mumps virus. Monkeys were infected under hexenal narcosis into Stenon duct of salivary glands, using a syringe with a special needle-cannula by injecting 0.5 ml of virus-containing liquid. The virus dose was 2×10^5—$2\times10^{5.5}$ HAU_{50}/1.0 ml of virus-containing liquid was injected intramuscularly in the right leg. Monkeys infected with different mumps virus strains were held in separate cages. Control animals were given placebo by the same route. Monkeys were observed for 21 days. Autopsy was done on days 3—5, 7—10, 19—21 post-infection (p.i.).

Virus isolation. Brain, spinal cord, parotid and submaxillary salivary glands, testes, lymphatic glands, pancreas and blood were examined. Homogenized, 20% suspension of organs and lysed blood were prepared in medium N-199 and centrifuged for 20 min at 3000 rev/min. Japanese quail embryo (QE) cell cultures grown in test tubes were infected with the supernatant liquid and incubated for 7 days at 37 °C. Irrespective of the results of the first passage, all specimens were tested in the second, and in doubtful cases — in the third passages. Specificity of the agents isolated was assayed in neutralization test (NT) with standard anti-mumps rabbit serum in QE cell cultures against 100 HAU_{50} of the Enders strain (Kaptsova *et al.*, 1976).

Mumps virus assay by immunofluorescence. Brain and parotid gland smears were fixed in acetone. Rabbit anti-mumps IgG labelled with fluorescein isothiocyanate (FITC) was used for the direct Coons method with bovine serum albumine; for the indirect method hyperimmune rabbit serum and FITC-labelled donkey anti-rabbit globulin were used. As controls, preparations were either treated with FITC-labelled IgG to vaccinia virus or the quenching phenomenon was used. Slides were examined in the microscope ML-2. Hyperimmune serum was raised in rabbits as described by Kravchenko *et al.* (1977).

Histological studies. Neural tissue was fixed in 10% formalin, salivary glands — in Bouin's fluid. Paraffin sections were stained with haematoxylin and eosin and with Nissl's stain.

Results

Histological and virological findings

No clinical symptoms indicating lesions of CNS and salivary glands were observed in 33 infected monkeys or in controls (Table 1). NA were found in all infected animals 19—21 days p.i., but not in the sera of control monkeys. The initial pathological changes in salivary glands such as serous exudate in the acini and swelling of the glandular epithelium were seen in monkeys infected with the L-3(7) strain on day 3 p.i. No pathological changes were seen at this time in the CNS and in visceral organs.

On days 5—7 the serous inflammation in salivary glands increased. Mononuclear infiltrations appeared around the ducts of acinar lobules. Small foci of neuron destruction were observed in the nuclei of cerebral pedunculi in one of the monkeys examined. Accumulation of microglia and macrophages was seen at the site of neuron destruction (Fig. 1). In chorioid plexi of lateral ventricles, all monkeys had plasma- and haemostasis along with proliferation of interstitial cells in the stroma. The ependyma of lateral ventricles and chorioid epithelium cells were swollen, but proliferative changes beneath the ependyma were moderate.

On the 10th day p.i. a marked destructive sialodenitis was observed in salivary glands (Fig. 2). In the brain, ependymal proliferation in the ventricle wall and perivascular infiltration in chorioid plexi of lateral ventricles were seen.

Table 1. Examination of monkeys inoculated into parotid gland with different mumps virus strains

Strain	Virus dose HAU	Day of sacrifice	Pathological changes		Virus detection in		Antibody response*
			chorioid plexus; ependyma	neurons	parotid gland	CNS	
L-3(22)	400 000	5	2/2**	0/2	0/2	0/2	0/2
		9	1/1	0/1	0/1	0/1	0/1
		19	2/2	0/2	0/2	0/2	22.6; 38.0
L-3(7)	600 000	3	0/2	0/2	2/2	0/2	0/2
		7	2/3	1/3	3/3	0/3	0/3
		10	1/2	0/2	1/2	0/2	0/2
		20	2/2	1/2	0/2	0/2	109, 11
Sofia-40	200 000	7	3 3	1/3	3/3	0/3	0/3
		20	2/2	0/2	n.t.	n.t.	8, 32
JL	200 000	3	3/3	0/3	2/3	0/3	0/3
		5	4/4	0/4	0/4	0/4	0/4
		10	2/2	0/2	n.t.	n.t.	n.t.
		20	3/3	0/3	0/3	0/3	64.8
Control		28	0/2	0/2	0/2	0/2	0/2

* Titres expressed as dilution reciprocals.
** Nominator: number of monkeys displaying pathological changes; denominator: total number of examined animals.
n.t. = not tested.

On day 20 the destructed parenchyma in salivary glands was substituted by connective elements. Lymphohistiocytic infiltrations were seen around ducts and vessels. Moderate chorioplexitis and ependymitis were observed in the brain of monkeys. In addition, one monkey showed discrete scattered nodular infiltrates at the site of neuron loss. Virus was detected only in the parotid gland in all animals killed on days 3—7 p.i.

Infection with L-3 (22) variants resulted in serous sialadenitis of the parotid gland on the day 5 p.i. Moderate chorioplexitis and ependymitis were seen in the CNS. On the day 9 minute foci of productive and proliferative sialodenitis were observed in salivary glands (Fig. 3). At this time no aggravation of previously seen changes was observed in the brain. Periductal infiltration was found in salivary glands; in CNS, focal proliferation of arachnoid cells and activation of pericytes around vessels were found on the external surface of hemispheres on day 19 p.i.

Virological examination revealed virus only in the parotid gland of one monkey killed on day 5 p.i. (Table 1). Infection with strain S-40 resulted in destructive infiltrative changes in parotid and submaxillary glands on day 5. p.i. The character of these changes was similar to those observed with the L-3(22) variant. Slight infiltrative and proliferative changes were observed in chorioid plexi and pia mater of the CNS in all monkeys (Fig. 4). Nodular proliferative lesions were detected below ependyma. However, no

Table 2. Pathological changes and detection of virus antigen in the brain of monkeys infected with different mumps virus strains

Strain	Virus dose HAU	Inoculation site	Number of monkeys	Days of observation	Pathological changes in		IF detection of virus antigen
					Ependyma, chorioid plexi, meninges	neurons	
L-3(22)	400 000	salivary gland	15	3	—	—	—
				5	+	—	+
				7	+	—	+
				10	+	—	—
				12	+	—	+
				14	+—	—	+
				19	—	—	—
L-3(22)	400 000	muscle	6	3	—	—	—
				5	+—	—	+
				7	+	—	—
				10	+—	—	—
L-3(7)	600 000	salivary gland	8	3	+	—	+—
				5	+	—	+
				7	+	+	+
				10	+	+	+
				18	+—	—	+—
JL	≥10 000	salivary gland	9	3	+—	—	—
				5	+	—	+
				10	+	—	—
				12	+	—	+
				14	+—	—	+—

encephalitis such as observed in monkeys infected with the L-3(7) was found. On day 21 periductial sialodenitis was seen in salivary glands and mild ependymitis in the CNS. Virological examination of monkeys carried out on day 7 revealed virus in the parotid gland in all 5 monkeys, but in the testis of one monkey only.

Infiltrative productive sialodenitis was detected in salivary glands in monkeys infected with "Jeryl Lynn". Periventricular gliosis and focal chorioplexitis were detected in the brain on the 5th day, but the destructive changes in chorioid plexi and ependyma were absent. Later on, the pathological changes did not increase, on the days 10—14 mild signs of glial proliferation were seen in the walls of lateral ventricles along with activation of pericytes in the stroma of chorioid plexi. Virological examination showed the presence of virus in parotid gland only, in two of three animals on day 3 p.i.

IF examinations

Failure to detect infectious virus in the CNS of monkeys led us to use immunofluorescence for determination of virus antigen in the brain. 38 monkeys were examined at different intervals p.i. (Tables 2, 3). It should be noted

Table 3. IF detection of virus antigen in the CNS of monkey infected intraductally and intramuscularly with different mumps virus strains

Days p.i.	IF intensity			
	Infection to salivary gland			Intramuscular infection
	L-3(7) strain	L-3(22) strain	JL vaccine strain	L-3(22) strain
3	+—	—	—	—
5	+	+	+	++
7	++	++	n/t	+
10	+	+	—	+—
12	+	++	—	n/t
14	+	+—	—	n/t
18	+—	—	n/t	n/t

(+—) = weak fluorescence in single cells; (+) = intensive fluorescence in 50% of cells; (++) = in 95 % of cells.
For further explanations see legend to Table 1.

that ependymal cells dominated in brain smears, the amount of neurons being small.

In brain smears of monkeys inoculated with L-3(7) strain focal fluorescence in single ependyma cells was seen on the day 3. On day 7 the maximum immunofluorescence was observed practically in all ependyma cells and in some neurons. Virus antigen was detected in a lesser amount of ependyma cells on day 10, but the intensity of fluorescence was on the same high level. Antigen was found in the brain smears until day 18 p.i. (Table 2).

In monkeys inoculated with the L-3(22) strain, virus antigen in ependyma cells was seen only on the 5th day p.i. The antigen was detected in the majority of cells on day 7, but not on the 10th day. By day 12 distinct granular cytoplasmatic fluorescence appeared again in a significant number of ependyma cells. On the 14th day p.i. antigen was detected only in a very limited number of cells and on the 18th day it was not seen at all (Figs 5, 6).

Intramuscular infection

Pathological changes in the CNS such as activation of astroglia and perivascular infiltration were observed in the periventricular zone of lateral ventricles. Specific antigen was detected by IF method in brain smears since day 5. Later on the intensity of IF slightly decreased and on the 10th day the antigen was seen in a few ependyma cells only (Table 3). In the cytoplasma of acinar cells of salivary glands the antigen was observed by 5—7 days p.i.

Discussion

Comparative morphological and virological study of neurovirulence and invasiveness of different mumps virus strains was carried out in monkeys after peripheral infection. Mumps virus strains penetrated into the brain

irrespective of their attenuation degree, causing pathological changes in its structure. However, the character and dynamics of pathological process depended on the degree of attenuation. In the CNS all strains under study caused perivascular nodular infiltration and pericyte activation around vessels in the wall of lateral ventricles. In addition, small focal destruction of neurons was observed upon infection with partially attenuated strains. The results obtained confirm our earlier suggestion (Yuzepchuk *et al.*, 1975) on neurotropism of wild strains and its loss in the course of attenuation.

Though pathological changes were found in the CNS of monkeys infected intraductally with mumps virus, no virus reproduction in the brain was detected. The specificity of morphological changes in the CNS, however, was proved using IF method. The negative results of virus detection in brain tissue using cell cultures may apparently be due to non-coincidence in localization of virologically examined regions with the foci of specific lesions in different brain areas. Results of IF revealed virus antigen in CNS in agreement with histological changes. Involvement of neurons into the pathological process in CNS of monkeys infected with the partially attenuated strains was proved by detection of specific IF in neurons.

Our previous experiments showed that the mumps virus vaccine strains were loosing their neurotropic qualities in the process of attenuation. But they retain neurovirulence consisting in the capacity to reproduce locally and cause lesions in the wall of brain ventricles.

Of importance is our finding of the invasiveness of mumps virus vaccine strains. They also retained the tropism to ventricle ependyma and epithelium cells of chorioid plexi after peripheral virus inoculation. This residual neurovirulence can be responsible for clinical symptoms in certain vaccinated persons manifested only with the mass mumps vaccination campaignes.

We found that the coincidence of viral antigen occurrence in the CNS with the manifestations of pathological lesions in brain tissue did not depend on the vaccine strain used or on the route of inoculation. Thus, not only wild and partially attenuated strains, but also vaccine strains may cause generalized process in monkeys infected intramuscularly and into the Stenon duct. However, highly attenuated strains loose their neurotropic qualities, although they are able to invade the CNS. Revealed differences between strains under study can serve as additional criteria of mumps virus attenuation.

Moreover, the generalization of pathological process after peripheral mumps virus is inoculated, may be used for analysis of postvaccinal complications in practice.

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Explanation of Micrographs (Plates XIV—XVII):

Fig. 1. Midbrain of monkey infected with strain L-3(7), day 7 p.i. Loss of single neurons, focal nodular infiltration in the nuclei of nervus oculomotorius. Nissl's stain. × 170.

Fig. 2. Parotid gland of monkey infected with strain L-3(7), day 10 p.i. Round cell infiltration around the duct of the gland and parenchymal destruction. Haematoxylin and eosin. × 170.

Fig. 3. Parotid gland of monkey infected with strain L-3(22) on day 9 p.i. Proliferation of stromal elements and lymphoid infiltration around the duct of the gland. Haematoxylin and eosin. × 200.

Fig. 4. The brain of monkey infected with strain S-40 on day 7 p.i. Neuronal loss and mononuclear infiltrates in corpus trapezoides. Nissl's stain, × 380.

Fig. 5. Mumps virus antigen in the brain smears of monkeys infected with the L-3(7) strain
I — virus-specific IF in ependyma cells; 5 p.i., × 490;
II — virus-specific IF in ependyma cells; day 7 p.i., × 490;
III — virus-specific IF in a neuron; day 7 p.i., × 490;
IV — virus-specific IF in ependyma cells; day 10 p.i., × 490.

Fig. 6. Mumps virus antigen in the brain smears of monkeys, infected with the L-3(22) strain
I — positive ependyma cells, day 7 p.i., × 490;
II — negative ependyma cells, day 10 p.i., × 490;
III — positive ependyma cells, day 14 p.i., × 490;
IV — ependyma cells stained with serum to vaccinia virus, day 5 p.i., × 490.