

EFFECT OF IMMUNOSUPPRESSION ON MORPHOLOGICAL CHANGES IN CNS OF MONKEYS INFECTED WITH DIFFERENT MEASLES VIRUS VACCINE STRAINS

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Summary. — Residual neurovirulence of measles virus vaccine strains “Leningrad-16”, “Moscow-5” and of cloned variant of L-16 strain — clone 3, differing in the degree of attenuation, was studied in intracerebrally (i.c.) infected *Cercopithecus aethiops* monkeys, receiving cortisone and/or antilymphocyte serum (ALS). Whereas the sensitivity for detection of the potential neurovirulence of vaccine strains was not increased by cortisone treatment, ALS had such effect in detection of neurotropic properties of the L-16 strain and its cloned variants.

Key words: measles; immunodepressants; neurovirulence

Introduction

In 1977 Shteinberg and Gordienko have reported on genetic characteristics of the clones, obtained from measles virus strain L-16. Their genetically homogeneous clone variants were characterized by morphological neurovirulence test in monkeys. Two clone groups differing in the neurovirulence test were determined as follows: 1) clones that caused neuron destruction and diffuse proliferative gliosis; 2) clone variants causing reversible changes in the area of injection track only. The vaccine strain designated “Moscow-5” (Sharova *et al.*, 1979) was chosen from the second group. It was important to obtain the comparative characteristics of vaccine strains under conditions of suppressed immunity. Two series of experiments were carried out using cortisone and ALS. The use of cortisone was foreseen to control neurovirulence of the measles virus vaccine strains by the U.S.A. Code of Federal regulation (1977). ALS was not used earlier to evaluate neurovirulent properties of measles virus vaccine strains.

Materials and Methods

Virus. One of the seed lots of measles virus strains L-16 was used as vaccine strain. “Moscow-5” strain was obtained by 3 times repeated cloning of the L-16 strain in Japanese quail embryo cell cultures. It maintained high immunogenic activity, was lacking of neurovirulent properties for monkeys and was genetically homogeneous. Clone 3 from the strain L-16 showed neurovirulent properties in i.c. infected monkeys (Shteinberg, Gordienko, 1977).

Table 1. Comparative morphological characteristics of CNS lesions in immunosuppressed monkeys infected with different measles virus vaccine strains

Strain (variant)	No. of monkeys	Interval of exam- ination (days)	Immuno- suppres- sion with	Morphological changes			
				neuron destruc- tion	diffuse gliosis	inflam- matory reaction	regen- erative changes
"Moscow-5"	4	28—30	cortisone	0/4*	0/4	0/4	4/4
L-16	4	28—30	cortisone	0/4	0/4	4/4	4/4
Clone 3	4	28—45	cortisone	4/4	4/4	4/4	0/4
"Moscow-5"	4	21—24	ALS	0/4	0/4	4/4	4/4
L-16	4	21—24	ALS	4/4	4/4	4/4	0/4
Clone 3	4	21—24	ALS	4/4	4/4	4/4	0/4

* Numerator = number of monkeys with CNS lesions; denominator = total number of monkeys

Immunosuppression. Domestic hydrocortisone was injected once intramuscularly (i.m.) at the time of virus inoculation in a dose of 200 mg/kg. The amount of cortisone corresponded to that stated by U.S. Federal requirements to measles virus vaccine control of neurovirulence.

ALS was injected i.m. one day before virus inoculation, simultaneously with it as well as 3 and 5 days post-infection (p.i.), i.e. altogether 4 injections each in a dose of 240 mg protein per kg body weight. ALS was obtained from Moscow G. N. Gabrichevski Institute of Epidemiology; its effect was checked in monkeys by counting of lymphocytes in peripheral blood 6 hr post-inoculation.

Animals. The experiments were carried out on green marmosets (*C. aethiops* weighing 2.0—2.5 kg, in which the presence of measles antibodies was excluded by haemagglutination-inhibition (HI) test. The monkeys were inoculated into thalamus at both sides with a dose of 1585 TCID₅₀ in 0.5 ml vol. Animals were sacrificed under deep hexenal narcosis on day 21—45 p.i.

Histological studies. The brain and the spinal cord were fixed in 10% formalin. Paraffin sections were stained with haematoxylin and eosin (HE) and with Nissl's stain. To reveal astroglial cells, Kanzler's method was performed.

Results

Cortisone treatment

The brain and the spinal cord of cortisone-treated animals, infected with "Moscow-5" strain, were examined morphologically. No changes of neurons and glial elements were observed in the cortex of hemispheres, midbrain, medulla oblongata and spinal cord. The glial scar seen in thalamic nuclei was in the stage of reparation (Fig. 1). Neurons of the scar zone showed axonal reaction characterized by central tigrolysis and nuclear displacement to the periphery of the cell. Examination of the pia mater revealed in some animals insignificant infiltration in the area of virus injection. Pathologic changes were not found in the ependyma cells, chorioid plexi of brain ventricles and in vessels of the brain parenchyma.

Neuronal damage and glial changes were not in detected monkeys infected with L-16 strain. Marked inflammatory reaction was observed in the late intervals. The reaction was most extensive in the injection scar area,

where mononuclear cuffs containing lymphocytes, macrophages and granular material were observed also in the late intervals of examination (Fig. 2, Table 1).

Encephalomyelitis with focal neuron lesions was seen in the brain and the spinal cord of monkeys, infected with the clone 3. The intensity of the inflammatory reaction drew our attention to these monkeys. In addition to glial elements forming the scar, abundant perivascular cuffs were found consisting of mononuclear cells and granular debris (Fig. 5).

Treatment with ALS

Morphological examination of the brain and spinal cord of ALS-treated monkeys infected with "Moscow-5" strain, showed the foci of accumulation of glial cells around neurons, which showed reversible changes. Diffuse gliosis was not seen outside the peritraumatic zone; scattered perivascular microinfiltrates were seen in the scar area (Fig. 4). In monkeys infected with the L-16 strain microfoci of neuronal destruction and single foci of neuronophagy were seen in the peritraumatic zone. Marked inflammatory reaction with vasculitis was found not only in the peritraumatic zone but widely outside it (Fig. 5). Encephalitis with numerous confluent foci of neuronophagy in the brain stem, intensive diffuse proliferative gliosis and extensive vasculitis in the brain parenchyma was observed in monkeys infected with the clone 3 (Fig. 6, Table 1).

Discussion

Comparative studies with two vaccine strains of measles virus (L-16 and "Moscow-5") and the variant clone 3 differing in the degree of their neurovirulence were carried out in monkeys. In our experiments, treatment with cortisone had no effect on the manifestation of neurotropic properties of the strains under study. Because of the absence of relevant publications we had no opportunity to compare our results with the corresponding neurovirulence control of measles virus vaccine strain in the U.S.A.

Since we were dissatisfied with the results of immunosuppression by cortisone in revealing of neurotropic properties of measles virus, we decided to look for other, more active drugs in order to suppress immunity to measles virus. Therefore we used ALS to display the potential neurovirulent properties of strains under study. In these experiments aimed at suppressing cell-mediated immunity, it was shown that "Moscow-5" strain lacked any neurotropic properties, even under the control conditions. In contrast, in monkeys inoculated with the clone 3, CNS revealed the same pathomorphological changes as seen in measles encephalitis (Allison, 1976).

Results obtained with animals infected with one of the seed lots of L-16 strain are of great interest. The residual neurovirulence of the vaccine strain was clearly demonstrated under conditions of suppression of cell-mediated immunity by ALS. We should point out that neurovirulent properties of the commercial measles vaccine strain were never detected earlier in control tests carried out at vaccine production. In addition, the pathological process

induced in the CNS of monkeys by different attenuated measles virus strains was characterized under conditions of immunosuppression by ALS.

It is known from medical practice that patients with congenital hypo- or agammaglobulinaemia recover from measles as usual and develop long lasting immunity, although specific antibodies are present in extremely low or undetectable levels. However, measles infection is always fatal in persons with congenital defects of the cell-mediated immunity or if it is artificially suppressed during long-lasting treatment with immunodepressants (Valdimarsson *et al.*, 1974; Ruckdeschel *et al.*, 1975; Aicardi *et al.*, 1977). All these clinical observations prove the paramount importance of the cell-mediated immunity in recovery and resistance to measles infection.

The advantage of testing neurovirulence of attenuated measles virus strains in monkeys receiving ALS, is in the high sensitivity of the method evaluating neurovirulent properties of vaccine strain candidates. At the same time it was found that properties of "Moscow-5" strain most completely meet all safety requirements.

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Explanations of Micrographs (Plates XXIV—XXVI):

- Fig. 1.* Brain of monkey, infected with strain "Moscow-5"; clearly delineated needle track without inflammation. 28 days p.i., HE, $\times 200$.
- Fig. 2.* Brain monkey, infected with strain L-16. Inflammatory reaction in the scar area. 28 days p.i., HE, $\times 200$.
- Fig. 3.* Brain of monkey, infected with clone 3. Extensive inflammatory reaction, consisting of lymphocytes and macrophages in the scar area. 28 days p.i., HE, $\times 200$.
- Figs 1—3.* Immunosuppression with cortisone.
- Fig. 4.* Brain of monkey, infected with strain "Moscow-5". Nodular infiltration in the region of necrotic neurons, mononuclear perivascular infiltration. 21 days p.i., HE, $\times 200$.
- Fig. 5.* Brain of monkey, infected with strain L-16; confluent foci of neuronophagy and vasculitis. 21 days p.i., HE, $\times 200$.
- Fig. 6.* Brain of monkey, infected with the clone 3. Confluent foci of neuronophagy with marked perivascular infiltration. 21 days p.i., HE, $\times 200$.
- Figs 4—6.* Immunosuppression with ALS.