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COMPARATIVE CLINICO-MORPHOLOGICAL INVESTIGATIONS OF THE CNS OF MONKEYS AND OF THE EYE TEGUMENTS OF GUINEA PIGS INFECTED WITH DIFFERENT MEASLES VIRUS STRAINS

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Summary. — Measles virus strains Edmonston and L-16 have been studied in 10 intracerebrally (i.c.) infected monkeys and in 155 guinea pigs infected into the anterior eye chamber. The strains appeared to differ in pathogenicity for monkeys and guinea pigs. The more pathogenic Edmonston strain caused encephalitis in monkeys, whereas in guinea pigs it caused iridocyclitis, keratoconjunctivitis and follicular conjunctivitis. Strain L-16 was not neurovirulent for monkeys, while in guinea pigs it caused follicular conjunctivitis. Specific pathohistological changes detected in the CNS of monkeys and in the eye teguments of guinea pigs were confirmed by virologic and serologic findings.

Key words: measles virus; iridocyclitis; follicular conjunctivitis

Introduction

At present, no laboratory markers of measles virus attenuation are available. The safety of measles virus vaccine strains is assessed by neurovirulence test for monkeys upon i.c. infection. At the same time, the damage of the eye conjuctiva is a constant clinical sign of measles infection extensively discussed in the literature (Zhaboedov and Shupik, 1975; Svastikova, 1970; Maichuk, 1981). However, morphological investigations of the eye teguments were carried out only by Dekkers (1981) who studied the cornea and conjunctiva of measles patients.

We have previously proposed an experimental model for differentiation of influenza virus strains according to their pathogenicity for guinea pigs inoculated into the anterior eye chamber (Rozina *et al.*, 1987). Pathologic changes in the eye teguments occur in monkeys inoculated into the anterior eye chamber with picorna- and ECHO viruses (Khoroshilova-Maslova and Polin, 1982; Koroleva *et al.*, 1984). The goal of the present investigation has been to design an experimental laboratory model (in addition to monkeys) for differentiation of the pathogenic properties of measles virus strains. Based

on clinical incidence of the eye damage in natural measles infection and on positive results obtained in guinea pigs infected with different influenza virus strains, we compared the pathohistologic changes elicited by measles virus strains of different pathogenicity. This was assessed on the basis of morphologic changes in the CNS of monkeys after i.c. inoculation and in the eye teguments of guinea pigs inoculated into the anterior eye chamber.

Materials and Methods

Viruses. Strain Edmonston (Enders and Peebles, 1954) was passaged in L-41 cells. Cell clone J-96 was derived from leukocytes of a leukaemic patient (Osgood and Brocke, 1955) and the clone L-41 was preparated in the Moscow Research Institute of Viral Preparations (Soloviev and Gulevich, 1960). The virus titre was $10^{5.7}$ TCD_{50}/0.5 ml.

The vaccine strain L-16 was isolated in 1960 in the Leningrad Pasteur Institute of Experimental Medicine; it underwent 21 passages in the guinea pig kidney cell culture (GPK) and 7 passages in the quail embryo cell culture (QE). The virus titre was $10^{5.3}$ TCD_{50}/0.5 ml.

Infection of animals. Guinea pigs weighing 300—350 g were given into the anterior eye chamber 0.1 ml virus after removing equal amount of fluid. The animals were examined with stereomicroscope at magnification 15×. Control guinea pigs (25) received the vaccine solvent by the same manner. Altogether 50 animals were infected with the Edmonston strain and 80 ones with vaccine strain L-16. The animals were observed for 21 days; they were examined on days 5, 7, 10, 14 and 21 post-infection (p.i.). At these intervals histological and virological examination of the eye teguments and serological test were performed.

Neurovirulence was examined in green monkeys after preliminary testing of serum for the presence of measles antibodies. The monkeys were inoculated with virus-containing material (0.5 ml) into thalamus opticus by a conventional procedure. Two monkeys were infected with the strain Edmonston and 8 were administered the strain L-16. The animals were sacrificed under hexemal anesthesia and bled on day 26 p.i.

Histologic examination. By the end of experiment, the enucleated guinea pig eye was fixed in 10% formalin and embedded into paraffin. Sections 8—9 μm thick were stained with haematoxylin-eosine (HE). The brain and spinal cord of monkeys was fixed in 10% formalin. Paraffin sections 7 μm thick were stained with HE and according to Nissl.

Virus isolation. Guinea pig eye was homogenized and 10% suspension was prepared in 0.85% sodium chloride solution. It was centrifuged at 1500 rev/min for 10 min. The supernatant was inoculated at 0.2 ml aliquots into 4 test tubes seeded with L-41 cells 24 hr before. The test tubes contained 0.8 ml of medium 199 with 10% bovine serum. Four test tubes with uninfected cell culture were left for control. The cultures were incubated at 37 ± 1 °C. The results were read on day 5 and then on day 10 according to cytopathic effect (polykaryocyte formation).

Serologic examination. To determine the antibody response in guinea pigs blood was taken on days 7 or 8, from monkeys on day 26. The sera were inactivated at 56 °C for 30 min and tested in haemagglutination inhibition (HI) test.

Results

Edmonston strain

Clinical evidence indicated that the earliest pathologic signs appeared on day 2. At that time, diffuse corneal opacification, changes of the iris colour and pericorneal vascular injection have been observed. On days 3 to 4 vascular injection round the limbus appeared, corneal changes became mostly focal and were often associated with an inflammatory process in the conjunctiva. On days 10 to 12 all animals had only insignificant changes of the iris colour and by the end of the 3rd week clinical signs did not differ from those in mock-infected animals.

Table 1. Detection of different measles virus strains in the eye teguments of guinea pigs

Strain	Sample no.	Days p.i.					
		4	5	6	7	8	9
Edmonston	1	−	+	+	+	−	+
	2	−	+	+	+	−	+
	3	−	+	+	+	−	+
	4	−	+	+	+	−	+
	5	−	−	+	−	−	+
	6	−	−	+	−	−	+
	7	−	−	+	−	−	+
	8	−	−	+	−	−	+
Measles vaccine L-16	1	−	−	+	+	+	−
	2	−	−	+	+	+	−
	3	−	−	+	+	+	−
	4	−	−	+	+	+	−
	5	−	−	+	+	−	−
	6	−	−	+	+	−	−
	7	−	−	+	+	−	−
	8	−	−	+	+	−	−

Note: + presence of the virus as detected in the lst passage.

Microscopic examination by week 1 showed diffuse fibrinous exudation in the iris and the ciliary body. The stroma was infiltrated with mononuclear cells and, to a lower extent, with plasma cells (Fig. 1). Chromatophores of the iris and the ciliary body were partially destroyed, as well as the pigmented epithelium. The iris stromal vessels were dilated and surrounded with lymphoid accumulations. Connective tissue and lymphoid cell accumulations as well as single neutrophil elements were detected in corneal stroma.

By week 2 formation of multiple lymphoid follicles (Fig. 2) and of multinuclear syncytia (Fig. 3) was detected in subconjunctival tissues. Signs of exudation in the iris abated. By week 3 only residual infiltration of the iris and ciliary body stroma were observed.

Vaccine strain L-16

Clinical examination on day 2 showed slight conjunctival vascular injection and a local corneal opacification. The iris colour changed insignificantly. In most animals vascular conjunctival injection persisted by days 4 or 5; it was associated with slight inflammatory changes in the semilunar fold and local corneal opacification. By days 10 to 14 only posttraumatic regenerative changes were found in the cornea. Microscopic examination carried out during the first week of the experiment indicated local destruction of the corneal stroma with concomitant inflammatory infiltration of the stromal vessels in the iris and ciliary body (Fig. 4). By week 2 single chromatophores

appeared destroyed in the iris. By the end of week 3 the changes in the eye tissues of infected animals did not differ from controls.

Controls

Clinical examination of most animals by days 3 or 4 of the experiment showed insignificant posttraumatic corneal changes and local vascular injection around the limbus. These signs disappeared within 10 days. Microscopic examination revealed only destructive changes in a few pigment cells of the iris and posttraumatic changes in the cornea. No changes were observed in other regions of the eye.

CNS of monkeys

Morphologic examination of CNS of monkeys infected with vaccine strain L-16 has shown a linear glial scar with a sharp outline. No specific changes were observed in the brain or spinal cord.

In animals infected with the Edmonston strain a scar with a broad zone of proliferative gliosis had formed; giant multinuclear cells were found in this cicatrix zone (Fig. 5). There was no sharp boundary between the traumatic zone and the surrounding tissues. Focal destruction of neurons (Fig. 6) and vasculitis were seen in the peritraumatic zone. Vasculitis and perivascular infiltration were also found under the ependyma of the anterior horn of lateral ventricles.

Virologic and serologic examinations

Virologic findings indicated the presence of the virus in the eye teguments of guinea pigs on days 5, 6, 7 and 9 after infection with the Edmonston strain and on days 6, 7 and 8 after infection with the vaccine strain L-16. No virus reproduction was observed in control animals (Table 1).

Serological tests detected virus-specific antibodies in the serum of guinea pigs infected with either strains on day 7 or 8 of the experiment.

Discussion

Measles strains Edmonston and L-16 inoculated into the eye anterior chamber of guinea pigs elicited clinical and pathomorphological signs of follicular conjunctivitis and iridocyclitis. Strain Edmonston caused more diffuse and profound damage as compared with strain L-16, characterized by formation of multinuclear giant cells in the subconjunctival eye tissue. The differences in pathogenic properties of the strains tested were well visible at morphologic examination of the brain of infected monkeys. Strain Edmonston caused measles encephalitis with destruction of neurons, vascular endothelium and glia in the cerebral areas adjacent to the scar zone. On the other hand, infection with strain L-16 was not associated with any changes in the brain outside of the traumatic zone. Thus strain Edmonston was more pathogenic in both experimental models causing encephalitis in monkeys and

generalized damage of eye teguments in guinea pigs. Vaccine strain L-16, however, was not neurovirulent for monkeys and caused only short-lasting damage of the eye teguments which disappeared by the end of the experiment.

The results indicate that guinea pigs infected into the anterior chamber can serve as a tool for differentiation of measles virus strains with respect to their pathogenic properties. This can be used to assess the biologic properties of the strains. Furthermore, we have shown a direct relationship between the pathogenicity of the strains tested and their reproduction capacity. It is noteworthy that the more pathogenic Edmonston strain caused syncytium formation both in the nervous tissue of monkeys and in the eye teguments of guinea pigs, whereas up on infection with the vaccine strain L-16 polycaryocytes were detected neither in monkeys nor in guinea pigs.

Serologic findings have also confirmed the specificity of the pathologic process in the eye teguments of guinea pigs and in the CNS of infected monkeys. Shroit and Kozlyuk (1969) and Shroit (1970) used guinea pigs for testing the experimental measles vaccine; however, they failed to detect any interstrain differences on subcutaneous infection. The administration route proposed in the present paper seemed to increase the susceptibility of guinea pigs to measles virus infection.

Thus comparison of 2 measles virus strains at clinical, morphological, virological and serological examinations demonstrated the advantages of the proposed experimental model for the assessment of pathogenic differences among measles virus strains. This model can be employed for selecting new vaccine strains and for safety control of vaccines in industrial production.

However, a few strains have been tested so far, so that no final conclusion can be made. Further studies on freshly isolated measles virus strains are desirable.

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Legends to Figures (Plates XX—XXII):

Fig. 1. Mononuclear infiltration in the stroma of iris and ciliary body; magn. 360×; HE staining.
Fig. 2. Multiple lymphoid follicles in the subconjunctival tissue; magn. 270×; HE staining.
Fig. 3. Giant-cell syncytia in the subconjunctival tissue; magn. 360×; HE staining.
Fig. 4. Inflammatory infiltration of the iris vessels and ciliary body stroma; magn. 270×; HE staining.
Fig. 5. Formation of multinuclear giant cells in the scar zone; magn. 360×; HE staining.
Fig. 6. Focus of neuron destruction in the peritraumatic zone; magn. 360×; stained according to Nissl.