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PATHOMORPHOLOGIC CHARACTERIZATION OF CNS DAMAGE IN MONKEYS INFECTED WITH PERSISTENT VARIANT OF MEASLES VIRUS VACCINE STRAIN L-16

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Summary. — The lesions of CNS were examined in monkeys infected intracerebrally (i.c.) with a variant of measles virus vaccine strain L-16 isolated after prolonged persistence in human cell culture NEr-2. The persisting virus variant appeared pathogenic for monkeys. The changes which had developed in their CNS within 30 to 60 days post-infection (p.i.) were alike to acute measles encephalitis which was evidenced by giant cell formation at the injection site. Twenty-two months p.i. the chronic character of lesions was evident from the appearance of foci of neuron destruction. Based on morphologic findings it was suggested that strain L-16-II has acquired some properties characteristic of nonattenuated virus.

Key words: measles; persistence; neurovirulence; vaccine strains

Introduction

Measles virus is the aetiologic agent of subacute sclerosing panencephalitis (SSPE). At present, it is still not clear why the same pathogen in some cases causes acute infectious disease followed by complete recovery, while in other rarer cases a slow infection with fatal outcome develops. Mutations of the virus genome arising after acute disease may be the probable cause of measles virus persistence and development of SSPE.

It is known that in the course of persistence in cell cultures viruses change many of their properties owing to the mutation rate under cultivation conditions (Holland *et al.*, 1982). Therefore, it was of interest to study the pathogenicity for monkeys of measles virus vaccine strain L-16 which persisted for 2 years in the human cell culture NEr-2. The infected monkeys were observed for as long as 2 years. Measles virus vaccine strain L-16 and the clone Moscow-5 isolated from this strain have been used as controls. Strain L-16 has been genetically characterized and the properties of its clone variants have been tested by Shteinberg and Gordienko (1977) and Shteinberg and

co-workers (1978). Pathomorphologic changes in monkeys infected with the strain L-16 and with clone Moscow-5 at early intervals p.i. were characterized previously (Sharova *et al.*, 1979; 1984).

Materials and Methods

Virus. The monkeys were infected with virus strain L-16 (persistent variant) isolated from chronically infected cells (CIC) NEr-2 two years after the onset of infection. Comparative characterization of the vaccine strain L-16 and its persistent variant has been carried out by Andzhaparidze *et al.* (1986*a*, *b*). Four ml of Medium 199 have been added to the monolayer of CIC grown in one-liter flasks; then it was 3 times frozen and thawed, centrifuged at 3000 rev/min, and the supernatant fluid was used for inoculation of the animals. Measles virus vaccine strain L-16 and its cloned variant Moscow-5 were used in the form of culture fluid collected from infected Japanese quail embryo cells.

Animals. The studies were carried out in green monkeys with preliminary testing of blood sera for measles antibodies in haemagglutination inhibition (HI) test. Altogether 2 experiments on 34 seronegative animals weighing 1.5—2 kg have been made. From these, 17 monkeys were infected with strain L-16-II, 8 and 7 monkeys were infected with strains Moscow-5 and L-16, respectively. The viruses were administered into both thalami under hexenal anesthesia at following doses: persistent variant L-16-II — 1600 TCD_{50} and vaccine strains L-16 and Moscow-5 — 1585 TCD_{50} in a volume of 0.5 ml. The monkeys were, in addition, inoculated subcutaneously with 1 ml of each variant of the virus. The control animals were given into both thalami 0.6 ml of the material collected from uninfected Japanese quail embryo cell culture. The animals were autopsied under deep hexenal anesthesia 1, 2, 4, 6, 14, 16 and 22 months p.i.

Histologic examination. Brain and spinal cord were fixed in 10% neutral formalin. Paraffin serial sections were stained by haematoxylin-eosin and according to Nissl. Kantzler's method was used for staining glial cells.

Fluorescent antibody method. Brain smears and cryostat sections were stained with convalescent human serum diluted 1 : 8. Control brain preparations from uninfected monkeys which had not been in contact with tested animals were used, as well as brain preparations of control monkeys which were given culture fluid collected from uninfected Japanese quail embryo cells.

Results

No somatic or neurologic changes have been found in the animals throughout the observation time.

Encephalitis with foci of neuronophagia situated both in the area of needle track and outside has been seen within days 30 to 60 at morphological examination of the brain and spinal cord of monkeys infected with L-16-II. Foci of necrotic neurons were generally found close to the vessels, and were accompanied with inflammatory signs in the vascular wall. The changes at the site on injury varied from endothelial swelling and perivascular accumulation of round-cells to panvasculitis.

Production of syncytia in the site of injection of virus-containing material was a specific feature of this process. Single giant cells were registered on day 30 p.i. By the end of month 2 a considerable amount of giant multinuclear cells was observed at the injection site. Four months p.i. they were decomposed, and a large number of giant engorged oligodendroglial forms were found in the scarring area (Figs 1—2).

After 4 to 6 months, microfocal nodular encephalitis developed in midbrain and medulla oblongata; however, in addition to small foci of destruction,

formation of neuronophagic nodules was also observed. Inflammatory elements were still present in perivascular infiltrates. Diffuse gliosis was extended largely over regions laying beyond the peritraumatic area. Both, capillaries and larger vessels were considerably swollen.

After 14 months perivenous demyelination was predominant in the CNS. It was located in the internal capsule, corpus callosum, anterior white commissure and thalamus, i.e. in formations adjacent to the injection site of the virus material. Nodular foci of gliosis consisting of micro- and astrocytic elements were detected in the thalamic nuclei and pulvinar, to some extent in nc. caudatus, s. nigra and cerebral peduncles. No destruction of neurons was observed at this interval. Examinations at 17—22 months p.i. showed microfocal encephalitis in the brain stem; by the end of the experiment the process became more extensive. Microfoci of neuronal destruction were found, as a rule, in the vicinity of blood vessels. Glial nodules persisted in midbrain and medulla oblongata (Fig. 3).

These data point at neuropathogenicity of the persistent variant of L-16-II virus for monkeys.

Morphologic examination of the brain of monkeys infected with the vaccine strain L-16 by 2 months p.i. showed subventricular encephalitis with signs of necrosis and ventriculitis. Perivascular infiltrates were detected even around the pituitary infundibulum (Fig. 4). After 4 to 6 months the process was characterized by lowmarked hyperplastic response of glial elements in the peritraumatic area and formation of glial scars at the inoculation site of virus-containing material. No destruction of neurons was observed.

After 12 months the lesions had similar appearance. Moderate nodular astrocytic proliferation was present in brain stem. Insignificant focal demyelination was observed in the white matter of internal capsule.

Microfocal encephalitis was seen starting from month 17. Microfoci of neuron loss were seen mostly in thalamic nuclei and pulvinar, in s. nigra, in basal nuclei of the brain, especially in the striatopallidary system. Nodular gliosis has been also observed. Summing up, the vaccine strain L-16 of measles virus also revealed some residual neurovirulence.

The following changes were found at the inoculation site in the brain of 8 monkeys infected with strain Moscow-5: at early intervals p.i. the scarring area was distinctly separated from the surrounding tissue, and in the needle track itself regeneration process could be seen. No changes were observed on neurones or glial elements in cerebral cortex, midbrain and medulla oblongata. After 2, 4, 6 and 22 months p.i. slight glial scarring was observed, the reparative changes being consistent with the observation interval. Only in 1 animal nodular gliosis was found with the L-16, Moscow and L-16-II vaccine strains at peritraumatic area in the thalamus. We conclude that the Moscow-5 strain appeared to be virulent.

All attempts to isolate infectious virus from the brain of 10 monkeys infected with the L-16-11 variant at late examination intervals (12 months) failed either by inoculation of monkey brain suspension into L-41 cells (a cloned variant of J-96 cell culture) and into Vero cells, or by cocultivation of trypsinized brain cells with L-41 and Vero cells, or by explantation.

Brains of 11 monkeys infected with each variants of measles virus were examined at 2 and 22 months p.i. by immunofluorescence. Single cells with bright fluorescence in cytoplasm were detected by 2 months p.i. in the brain smears of monkeys infected with the L-16, Moscow-5 and L-16-11 vaccine strains. Specific fluorescence of single neurones far beyond the traumatic area and distinct cytoplasmic fluorescence of glial cells were seen in cryostat sections of each monkey by 22 months p.i. No fluorescent cells have been observed in the scarring area. It is noteworthy that some fluorescent glial cells were located in the vicinity of the vessels. Only a few fluorescent glial cells were detected in the cortical regions and in the white matter of hemispheres whereas the majority of positive cells was located in the thalamus, hypothalamus and in nucleus caudatus, i.e. in those brain regions which revealed histologic changes of neurones and glial cells (Figs. 5—10). No fluorescence of neurones or glial elements was observed in control preparations.

Serologic examination revealed antibodies to measles virus regardless of the time of testing.

Discussion

The data obtained indicate that the variant of measles virus vaccine strain L-16 isolated after prolonged persistence in human cell culture NEr-2 was pathogenic at i.c. administration. The pathologic process which has developed in the CNS of animals one month p.i. can be defined as acute encephalitis as confirmed by the formation of giant-cells at the virus injection site. Nevertheless, the acute stage was not terminated, the process taking a progressive course, probably due to the reproduction of the inoculated virus. For instance, by 4 to 6 months p.i. in addition to microfocal destruction of neurons, foci of neurophagia were noticed and perivascular cuffings could be seen.

The process had a cyclic nature, as after the remission of 11 months the picture of CNS damage reappeared. This was characterized by changes of glial elements in the form of diffuse and nodular gliosis, microfoci of neuronal destruction and demyelination. Evidently, the process had a chronic course.

The comparison of morphologic changes in the CNS described in SSPE patients (Buravtseva, 1971; Karmysheva *et al.*, 1974) with the experimental process observed in monkeys indicated similar character of the changes, but their topography was obviously different. Cerebral cortex of the brain appeared to be spared. Probably, even longer observation would allow to determine with more certainity whether or not the cortex would become affected. The development of pathologic changes in the CNS of monkeys infected with the vaccine strain L-16 by 2 years p.i. is another reason for more cautious evaluation of this process. With respect to morphology and localization of the damage, these animals were shown to have lesions similar to those observed in monkeys infected with the L-16-11 variant.

Summing up, it should be noted that histologic findings suggest that strain L-16-11 has acquired some properties characteristic of unattenuated virus.

Comparative studies on two vaccine strains of measles virus L-16 and Moscow-5 have shown that strain L-16 has a residual neurovirulence. The development of periventricular encephalitis, hyperplastic response of glial elements in brain stem formations and chronic encephalitis 22 months p.i. testify the doubtless neuropathogenicity of virus strains tested. Probably, this is related to genetic heterogenicity of measles virus strain L-16, which, in its turn, resulted in the selection of viral particles with higher pathogenicity in the population owing to a variety of factors of external and internal nature. It has been shown that strain Moscow-5 was not neurovirulent. Signs of astrocytic nodular gliosis have been observed after 22 months in 1 animal only.

Thus, our investigations have provided additional evidence that, in spite of attenuation, genetically heterogenous measles virus strain L-16 has retained its potential neurovirulence (Sharova *et al.*, 1983). Our data also indicated that further cloning promoted the production of strain Moscow-5 which seemed not neurovirulent for monkeys.

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Explanation to Figures (Plates LI—LIII):

Figs. 1—2. Thalamic lateral nucleus, scarring area, strain L-16-II. 1 — day 30: production of polykaryocytes in progress; 2 — day 60: polykaryocyte formation; stained with haematoxylin-eosin; magn. 1 — 140×, 2 — 400×.

Fig. 3. Pulvinar thalami; glial nodule at the site of neuron death (I); tigrolysis (II). Strain L-16-II, 22 months p.i., Nissl stain; magn. ×400.

Fig. 4. Nucleus caudatus, panvasculitis in subventricular zone, vaccine strain L-16, day 60 of the experiment, stained with haematoxylin-eosin, magn. ×280.

Figs. 5—7. Bright diffuse fluorescence in the cytoplasm 1 month p.i. 5 — vaccine strain L-16; 6 — strain L-16-II; 7 — strain Moscow-5.

Figs. 8—10. Perinuclear fluorescence of the cytoplasm 22 months p.i. 8 — L-16, 9 — L-16-II, 10 — Moscow-5.