LOCALIZATION OF SPECIFIC ANTIGEN IN THE ORGANS OF NEWBORN ANIMALS VACCINATED WITH LIVE SMALLPOX VACCINE

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Of 20 newborn rabbits aged 4-5 days vaccinated intradermally with live smallpox vaccine, six developed manifestations of generalized vaccinia and neuroparalytic complications. Intensive accumulation of the specific antigen in the brain, lungs, spleen and lymph nodes was demonstrated by the fluorescent antibody method. Vaccinia virus was isolated from the same organs. In 14 newborn rabbits killed while clinically healthy, long persistence of attenuated virus was observed in the brain, spinal cord, lungs, spleen, and lymph nodes. The specific antigen was detected by the immunofluorescence method. Vascular disturbances and slight cellular changes were observed in the brain tissue of the vaccinated animals. In sick animals these changes were more marked in character.

KEY WORDS: *immunofluorescence; vaccinia virus; persistence.*

Live smallpox vaccine is widely used in preventive medicine. Vaccination with live smallpox vaccine in some cases are accompanied by the development of postvaccinal complications, some of them neurological [1, 4].

The study of the penetration of the virus and the localization of the specific antigen and the pathomorphological changes in the CNS and organs of animals vaccinated intradermally are of considerable interest.

According to preliminary observations on newborn rabbits vaccinated with smallpox vaccine, vaccinia virus and the specific antigen could be detected in the brain, spleen and lungs of the animals for 20 days (the period of observation) [2].

The object of the present investigation was to study the distribution of vaccinia virus and the localization of its specific antigen and the pathomorphological changes in the brain tissue and internal organs of newborn rabbits vaccinated with live smallpox vaccine over a period of time.

EXPERIMENTAL METHOD

Experiments were carried out on 20 newborn rabbits aged 4-5 days. The animals were vaccinated intradermally in the thigh with a single human dose of commercial live smallpox vaccine produced by the Moscow Research Institute of Virus Preparations. On the 4th, 7th, 14th, 20th, 30th, 45th, 60th, and 90th days the rabbits were killed by total exsanguination, and the organs were then perfused with physiological saline through the systemic circulation. Cytoplasmic extracts were prepared from the organs of the exsanguinated animals, for vaccinia virus is known to reproduce in the cytoplasm of cells [5]. To obtain cytoplasmic extracts the organs were homogenized in 5 ml of 0.001 M Tris-buffer, pH 8.5, and low-speed centrifugation was carried out for 10 min at 3000 rpm followed by 30 min at 10,000 rpm. The residue was supended in 0.6 ml of 0.001 M Tris-buffer, pH 8.5, in a Downs' homogenizer, and again clarified at 3000 rpm for 10 min. The product was used as the original material and ascending dilutions were prepared from it up to 10^{-3}. The virus was isolated on primarily

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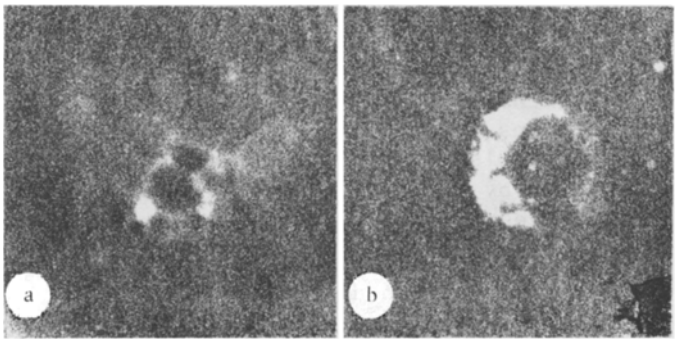

Fig. 1. Localization of specific antigen in organs of newborn rabbits on 14th day after smallpox vaccination: a) cytoplasmic localization of antigen in brain neuron; b) cytoplasmic localization of antigen in splenic plasma cell. Direct Coon's method, 720×.

trypsinized chick fibroblasts. The isolated virus was identified by neutralization and microprecipitation tests in the usual way.

For the microprecipitation test virus was accumulated by infecting chick embryos with culture fluid giving a total cytopathic effect, and this was followed by successive passages through 12-day chick embryos.

To prepare the cytoplasmic extracts squash preparations were made from the organs, fixed in cold acetone, and stained by the direct Coons' method, [3]. Pathomorphological changes in the tissues were studied in histological sections stained by the Romanovsky–Giemsa method in the usual way.

EXPERIMENTAL RESULTS

Of 20 vaccinated rabbits, six developed postvaccinal complications on the 7th-11th days after vaccination. The newborn rabbits developed generalized vaccinia, manifested as papular and vesicular eruptions of the abdomen, the sides of the trunk, and the footpads. In two animals generalized vaccinia was combined with neurological complications and one animal had neurological complications only. The remaining 14 animals were killed while clinically healthy at various times after vaccination.

In the clinically healthy animals the specific antigen of vaccinia virus was discovered starting from the fourth day after vaccination in the brain, spinal cord, lungs, lymph nodes and spleen. Vaccinia virus antigen was found in the brain and lungs throughout the period of observation – until the 90th day, in the spinal cord until the 45th day, and in the spleen until the 60th day. Detection of the antigen correlated with isolation of vaccinia virus from the organs. Fluorescence was most intensive between the 40th and 30th days. Antigen was found in the brain and spinal cord in the form of extracellular and intracellular granules, located in neurons and glial cells. In the spleen and lymph nodes the antigen was detected as cytoplasmic structures in macrophages, lymphocytes, and plasma cells (Fig. 1). Specific fluorescence also was found in the alveolar macrophages of the lung, and foci of homogeneous fluorescence of the tissue were frequently observed in the lungs. The intensity of fluorescence in the CNS and in the lungs diminished after 45 days, and in the spleen and lymph nodes after 30 days.

Fluorescence of the specific antigen was more intense in the CNS and internal organs of the sick animals. The brightest fluorescence, located in many neurons and glial cells, was observed in rabbits with neuroparalytic complications. Foci of homogeneous fluorescence were frequently found in the lungs of the sick animals. Vaccinia virus was isolated in all cases from the organs of the sick animals. Its titer was a little higher than in clinically healthy animals examined at the same time.

The method suggested by the writers for isolating virus from cytoplasmic extracts of organs, incidentally, is more sensitive than the usual methods of virological investigation and enables minimal quantities of virus to be detected. This method can be suggested for the virological study of lethal cases of post-vaccination complications developing after smallpox vaccination in man.

Slight disturbances of the hemodynamics were observed in the CNS on pathomorphological investigation of the organs of animals killed while clinically healthy in the early periods after vaccination.

By the 14th-30th days examination of the brain revealed the formation of perivascular cuffs, shrinking of single neurons, and pale cytoplasmic inclusions with an internal basophilic structure in them. In the later stages there were fewer shrunken neurons, the cytoplasmic inclusions were replaced by vacuoles, and the evidence of disturbance of the hemodynamics still persisted. Changes were less frequently found in the spinal cord.

In the lungs, in the early stages after vaccination swelling of the alveolar septa was observed, and from the 14th until the 30th days in some cases foci of pneumonia were present; these correlated with the discovery of homogeneous fluorescence of the tissue by the immunofluorescence method.

In rabbits with neuroparalytic complications damage to neurons was observed much more frequently. Damaged neurons were particularly numerous in the subcortical zone of the brain. Polymorphonuclear inclusions with a well-defined internal structure were found in the cytoplasm of the neurons in all cases.

In animals with generalized vaccinia, more severe changes were observed in the brain neurons and hemodynamic disturbances predominated. Signs of pneumonia were found in the animal's lungs.

The results of these investigations thus showed that vaccinia virus possesses well-marked neurotropic properties, which are manifested as the development of neuroparalytic complications in some animals and prolonged persistence of the virus in the CNS of rabbits killed while clinically healthy. The possibility of penetration of vaccinia virus into the CNS cannot be explained purely by the immaturity of the blood–brain barrier in newborn animals, for the writers' investigations on weaned rabbits also showed that vaccinia virus can penetrate into the CNS and persist therein.

It must be assumed that the pathomorphological changes observed in the brain are the result of the direct action of the virus on the CNS.

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