Vaccinia Virus Implicated in Diffuse Demyelinating Disease[1] (37417)

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The more recent association of the measles virus with late cases of encephalomyelitis (1–3) and the etiologic role of the measles virus in subacute sclerosing panencephalitis (SSPE) (4, 5) occurring years after childhood measles, have prompted the concept of the possible role of vaccinia virus as the causative agent of encephalomyelitis appearing years after a primary infection by this agent.

An association of encephalomyelitis with smallpox vaccination was brought to the attention of the medical community most forcefully by small epidemics of encephalomyelitis which appeared to have a direct relationship to vaccination. This complication of vaccination was most prevalent in Austria, England, Germany and Holland in the 1920's and early 1930's. As far as we know, the possible relationship of encephalomyelitis to vaccinia virus has not been previously recognized as appearing years after the primary vaccination. This possible association of vaccinia virus and subacute disseminated encephalomyelitis is based on virological, immunological and pathological evidence as well as a review of the literature which on several occasions refers to the similarities of the pathological findings in encephalitis caused by viruses such as measles, variola, vaccinia, and others (6–8). An additional objective of this report is to alert others to a possible causal relationship to stimulate further investigation of the problem.

The patient under study presents all of the classic features of neuromyelitis optica, both clinically and pathologically, and fits the description of Devic's disease or syndrome, first elaborated by Devic in 1894 (9). About 200 cases were reported in the literature up to 1950, varying in age from 5 to 60 yr (10).

Materials and Methods. The patient who is the subject of this report was followed intensively in the hospital for many months. Several samples of serum and spinal fluid were obtained for antibody studies, colloidal gold curves, total and fractionated protein determinations.

Post mortem examination was carried out within 2 hr following her demise to obtain material for tissue culture, fluorescent antibody studies, electron and light microscopy.

Vaccinia antibody titers were performed on sera and spinal fluid in Dr. Henry Kempe's Laboratory.[2] The method employed was vaccinia neutralization with and without complement enhancement. Fresh guinea pig serum was used as a source of complement with a titer of about 90 hemolytic units. Measles titers were obtained by use of the standard hemagglutination-inhibition method in this laboratory, and by the Virus and Rickettsial Laboratory of the California State Department of Public Health.

Tissues obtained within 2 hr of death were trypsinized and planted in T-60 falcon plastic flasks. Small explants were also placed in brain culture medium consisting of medium 199, fetal calf serum 20%, egg ultrafiltrate 10%, glucose 100 mg/100 ml. Antibiotics were added to the medium. Cocultures were established utilizing HeLa and human embryo kidney cells. Iododeoxyuridine and bromodeoxyuridine, 20 and 40 μg, and 2% dimethylsulfoxide were mixed with culture media. Cell cultures were treated with the

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mixture for 72 hr.

Brain tissues from grossly involved areas were frozen quickly in isopentane cooled with liquid nitrogen for fluorescent antibody (FA) studies. These tissues were cut and fixed in cold acetone overnight. A standard procedure was carried out by the indirect FA technique for evidence of measles or vaccinia antigen in brain tissue.

Three adult New Zealand white rabbits were inoculated subcutaneously with 1 ml at 4 sites on each of 3 occasions one week apart with material from the patient's brain emulsified with equal parts of Freund's adjuvant. Pre- and post-inoculation sera were tested for vaccinia antibodies by the plaque reduction and fluorescent antibody tests. Sera from ten uninoculated rabbits served as controls.

Routine histopathological studies of the patient's brain tissue were carried out employing hematoxylin-eosin, Luxol-Pas and Weil's myelin strains.

Results. This Negro girl was born on April 5, 1958, and died on November 26, 1971 at 3:30 AM, age 13½ yr. She was well until February 1969 when decreased vision was first observed. Four months later, blindness in the left eye with optic atrophy was diagnosed. In December 1969 ataxia and left facial numbness had developed. Mother stated that the patient had a stiff neck and a "wobbly" gait.

In February 1970 a diagnosis of multiple sclerosis was made in another clinic. ACTH therapy was started without success. The mother stated that our patient had numbness in the entire left side of her body from December 1969 to March 1970 when her condition began to improve and she returned to school. However, in April she had a fall and discontinued school in May. During June, July, and August, numbness became widespread in all extremities. Vision in the right eye decreased but improved in September to the point that she could watch television. In November 1970 vision again decreased and she was unable to roll over or sit up. Incontinence was observed for the first time.

Her menses started at age 9; however, she had had no menstrual period since the onset of the present illness. She was vaccinated against smallpox in 1958 at about the age of seven months. Diphtheria, pertussis, and tetanus immunization was also administered in 1958. She had measles early in 1959 at the age of 10 months. She received oral polio vaccination in 1968. Visual tests performed in 1968 were recorded as 20/20 in both eyes. A vaccination against smallpox was repeated in 1964 but no reaction was evident.

Physical examination. At the time of admission to the Rancho Los Amigos Hospital, her height was 55 in., weight 61½ lbs, blood pressure 92/58, pulse 80, temperature 94°F. Pupils were large and did not respond to light. The optic discs were pale. Nystagmus was present on lateral and upward gaze. Several pressure sores were present over the ankles and in the left trochanteric area. Neuromuscular examination showed no tone in the legs. Babinksi and Hoffman signs were present bilaterally. Reflexes in the upper extremities were brisk and tremor of the left upper extremity was observed. The sensorium was clear.

Laboratory findings. On December 15, 1970, hemoglobin 12.8, Hematocrit 38, WBC 4,500, Neutrophiles 67, Bands 2, Eosinophiles 9, Basophiles 1, Lymphocytes 16, and Mononuclears 5. Sedimentation rate 36, reticulocytes 2.6%. The spinal fluid was clear. An electroencephalogram performed on July 20, 1971 revealed a mild diffuse slowing pattern, greater over the right hemisphere. Colloidal gold, 4443333210, measles serum antibody titer by hemagglutination-inhibition (HI) test was 1:32. Repeated spinal fluid measles HI antibody titers were negative. Spinal fluid protein on the first examination was 224 mg/100 ml, sugar 41, white blood cells 18, all mononuclears. Spinal fluid protein determination subsequently varied from 78 to 200 mg/100 ml. Serum immunoglobulin A was reported at 100 mg/100 ml, gamma G immunoglobulin 1450 mg, and gamma M immunoglobulin 150/hundred ml.

On September 22, 1971 two months prior to death, the colloidal gold curve was 0111221000; total protein in CSF was 117 mg/100 ml, albumen 46%, globulin 54%, alpha 1, 7%, alpha 2, 5%, beta globulin 12% and gamma globulin 30% of the total

globulins. Vaccinia neutralization titers on sera and cerebro-spinal fluid (CSF) were markedly elevated. On 8 specimens of serum, titers ranged from 1:1280 to 1:5000 with complement and from 1:240 to 1:800 without complement. Titers on 5 specimens of spinal fluid ranged from 1:16 to 1:64 with complement over a 10 month period prior to her death in November 1971.

Post mortem examination. The brain weighed 1240 g, the optic nerves were atrophic and the optic chiasm revealed slight flattening with foci of yellow degeneration. There was melanin pigmentation over the upper cervical cord and ventral medulla. Multiple coronal sections revealed extensive confluent white matter disease (Fig. 1). A confluent destructive demyelinating process was found which involved subtotally the centrum ovale and extended into the occipital lobes, especially the optic radiation bilaterally. The cervical cord was similarly involved. The margins of the demyelinated process were poorly defined.

FIG. 1. A coronal section through the parieto-occipital region of the cerebrum showing extensive confluent white matter disease with necrosis and cavitation. The geniculo-calcarine radiation and the white matter subjacent to the calcarine cortex are especially involved. There is minimal secondary atrophy to the cortical strip. (Actual size).

Microscopic examination revealed subacute, focally cavitating demyelination in the cerebral hemispheres which includes frontal, parietal and occipital lobes, optic chiasm, brain stem and spinal cord. There was mild focal perivascular mononuclear cell aggregation. Numerous perivascular gitter cells were also apparent, and in some foci, especially in the basal ganglia, eosinophils were present around blood vessels. There was axonal destruction and only rarely was the demyelination associated with any preserved axons. Zones of subacute demyelination were present throughout the pons especially about the floor of the fourth ventricle and in the transverse pontine fiber tracts. In the brain stem there was sparing of the inferior olives but marked involvement of the periolivary white matter and external arcuate fibers. The optic chiasm was similarly involved. Inclusions similar to Guarnieri's bodies were observed in the cytoplasm of several neurons and one is illustrated in Fig. 2. These structures are negative for iron and calcium and have a slight "u" or comma shape and are not Nissl bodies. Study by electron microscopy revealed numerous lysosomes in damaged neurons and in reactive astrocytes about the edges of the demyelinated regions. Thus far, we have not isolated by electron microscopy good illustrations of the bodies seen in some neurons but we are continuing the search.

Brain tissue from the patient was emulsified with Freund's adjuvant and inoculated subcutaneously at 12 sites over a period of two weeks into three rabbits. The preinoculation sera from the rabbits was negative for vaccinia virus antibodies. Sera obtained at five intervals from 3 to 11 weeks postinoculation revealed elevated vaccinia antibody titers recorded at 1:48 in animals 1 and 2, and 1:120 in animal 3, tested by plaque reduction with complement. Titers on 8 specimens from 4 control rabbits in the same environment as the inoculated animals remain nega-

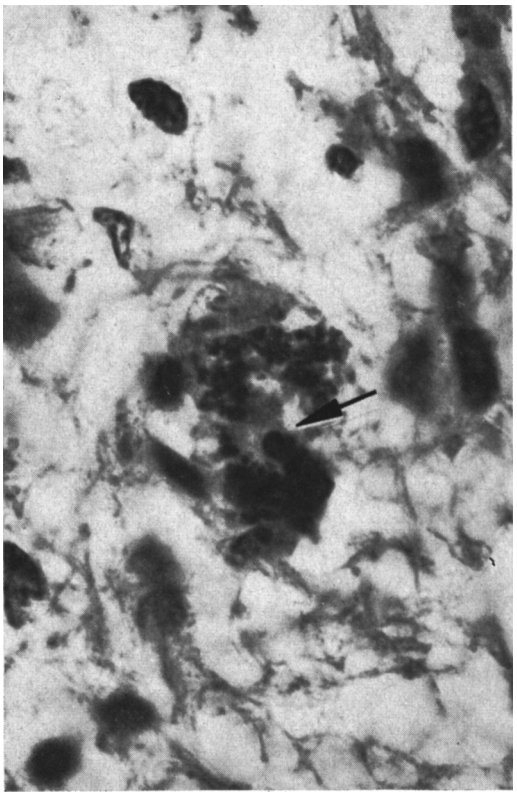

FIG. 2. One of several neurons found in the occipital cortex, which contained numerous comma or "u" shaped masses in the cytoplasm. These resemble light microscopic descriptions of Guarnieri or Paschen bodies. The nucleus and nucleolus (↑) of the cell is obscured. (×1600).

tive. Tests on total of 10 rabbits, including 3 preinoculation specimens, have all been considered negative.

The results of tissue culture studies as outlined under "Materials and Methods" were inconclusive and, therefore, we were unable to interpret them, and they must be considered negative. The fluorescent antibody studies on the material available were considered negative. We hope that this preliminary report will encourage and stimulate further research on this important problem.

Discussion. The patient died at the age of 13½ yr after an illness of approximately 33 months. Her illness was wholly compatible with the diagnosis of neuromyelitis optica as described by Ford (11). The syndrome of optic neuritis with subsequent atrophy and transverse myelitis is said to be more common in children than adults but may occur at almost any age according to Merritt (12). This patient received her primary vaccination against smallpox at approximately 6–7 months of age. A second vaccination at age 6 showed no reaction.

The clinical course of this patient was characterized by remissions and exacerbations. Her sensorium remained clear until the few weeks prior to death. Her body temperature was subnormal, averaging 94–96°F. It is of interest that Ford (11) records a single case of neuromyelitis optica in a nine-year-old girl whose temperature averaged between 90 and 100°F.

The gross and microscopic findings at autopsy showed strikingly severe involvement of the white matter of the brain and cord. Necrosis and cavity formation does occur in the severe forms of this disease (11). Greenfield (13) points out that thickening of vessel walls is commonly seen in old lesions and were present in our patient. She also had focal calcification about some blood vessels. Perivascular cellular infiltration was characteristic of viral encephalomyelitis. The extensive disorder of the myelin in the region of the optic radiation bilaterally immediately brings Schilder's disease to mind, but the involvement of the spinal cord and optic chiasm in addition to the extensive cystic alteration and necrosis of myelin speaks against this and points more toward the classic description of neuromyelitis optica.

It is interesting to record that Pierre Marie (14) in 1895 stated in referring to causation of demyelinating disease, "*Eruptive fevers* must also be mentioned, namely, *measles, scarletina* and, above all, *smallpox.* Cases are numerous in which insular sclerosis has been known to occur during convalescence after the latter affection: tremor in the limbs with more or less paresis, disorder of the speech which becomes slow and scanning, nystagmus and, in short, all the characteristic symptoms of insular sclerosis may exist. At times these symptoms cease and entirely disappear but they may also continue and confirmed insular sclerosis occurs."

Appleyard and Westwood (15), in studies on the formation of neutralizing antibodies in rabbits, reported a good response when

the antigen was given subcutaneously with adjuvant. They state, "In the absence of adjuvant, even a massive single dose of antigen given subcutaneously elicited very little antibody."

Summary. Virological, immunological and pathological data were obtained from a child who died at the age of 13½ yr, following an illness of 33 months. Her fatal illness may be related to infection by the vaccinia virus administered as a primary vaccination against smallpox at the age of seven months. Antibody titers against vaccinia virus of 1:5000 were found in her serum and several spinal fluid determinations revealed antibody titers from 1:16 to 1:64 when tested by the vaccinia neutralization test with and without complement. Rabbits inoculated with brain material plus adjuvant developed titers against vaccinia virus. Control animals were negative. The patient had a severe form of subacute disseminated encephalomyelitis with necrosis and cavity formation, characterized by perivascular infiltration, circumscribed and diffuse sclerosis.

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1. Adams, J. M., Baird, C., and Filloy, L., J.A.M.A. **195**, 290 (1966).

2. Adams, J. M., Neurology **18**, 52 (1968).

3. Adams, J. M., and Brown, W. J., Int. Arch. Allergy Appl. Immunol. **36**, 83 (1969).

4. Horta-Barbosa, L., Fuccillo, D. A., London, W. T., Jabbour, J. T., Zeman, W., and Sever, J. L., Proc. Soc. Exp. Biol. Med. **132**, 272 (1969).

5. Payne, F. E., Baublis, J. F., and Itabashi, H. H., New Engl. J. Med. **281**, 585 (1969).

6. Turnbull, H. M., and McIntosh, J., Brit. J. Exp. Pathol. **7**, 181 (1926).

7. Perdrau, J. R., J. Pathol. Bacteriol. **31**, 17 (1928).

8. Spillane, J. D., and Wells, C. E. C., Brain **87**, 1 (1964).

9. Devic, A., Congr. Francais Med. **1**, 139 (1948).

10. Stansbury, F. C., Arch. Ophthal. **42**, 292 (1949).

11. Ford, F. R., "Diseases of the Nervous System in Infancy, Childhood and Adolescence," Fifth Ed. p. 207. Chas. C Thomas, Springfield, IL (1966).

12. Merritt, H. H., "A Textbook of Neurology," p. 728. Lea and Febiger, Philadelphia (1968).

13. Greenfield, J. G., Blackwood, W., McMenemey, W. G., Meyer, A., and Norman, R. M., "Neuropathology," p. 456. Edward Arnold, Ltd., London (1961).

14. Marie, P., "Lectures on Diseases of the Spinal Cord," The New Syndenham Society, London (1895).

15. Appleyard, G., and Westwood, J. C. N., Brit. J. Exp. Pathol. **45**, 162 (1964).

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