Further Studies of Viral Antibodies in the Cerebrospinal Fluid of Patients with Multiple Sclerosis: Vaccinia and Parainfluenza Type 1 (37422)

PAUL BROWN, FRANCOISE CATHALA, AND D. CARLETON GAJDUSEK
(Introduced by J. A. Morris)

National Institute of Neurological Disease and Stroke, National Institutes of Health, Bethesda, Maryland 20014

In an earlier paper (1) we reported elevated measles antibody levels in cerebrospinal fluid (CSF) from a large series of patients with multiple sclerosis (MS) as compared to patients with other neurological diseases. Since our report, ter Meulen *et al.* (2) have isolated parainfluenza virus type 1 from brain tissue from 2 patients with MS, and Kempe (3) has found antibody to vaccinia virus in a high proportion of CSF specimens from MS patients, but not controls. Support of the parainfluenza virus isolations by antibody data, or confirmation of the vaccinia antibody study would considerably strengthen the implication that either virus could in at least some patients be causally related to MS. We have, therefore, gone back to our original series of CSF specimens, and report here the results of tests for neutralizing antibody to these viruses.

Materials and Methods. Specimens. CSF from 127 patients with MS and 78 patients with other neurological diseases were tested for antibodies to vaccinia virus. This is substantially the same series, with fewer controls, on which measles antibody titers were earlier reported, and is fully described there (1). CSF specimens from approximately one half of this series (62 MS and 36 control patients) were tested for antibody to parainfluenza virus type 1.

Antibody assays. Vaccinia virus, originating from the IHD strain (4), and grown in HeLa cells, was obtained from Dr. Samuel Baron, NIAID, NIH. Equal volumes of the undiluted CSF specimens were mixed with 600 pfu of sonicated vaccinia virus in Eagles # 2 medium, incubated with occasional agitation for 2 hr at 37°, and then further diluted 1:10 before 0.2 ml volumes were added to triplicate vertical 25 mm flat-bottom glass vials (5) containing sheets of confluent L-cells. Following a further incubation with occasional agitation for 1 hr at 37°, the vials were fed 2 ml of Eagles # 2 medium containing 2% fetal calf serum, and replaced at 37° in an atmosphere of air and 5% CO_2 (6). After 40–44 hr, the cells were washed, fixed, and stained with a 0.2% crystal violet in methanol solution, and the plaques counted under low power magnification. A 50% or greater reduction in plaque number relative to the virus control was considered to indicate the presence of antibody in the tested specimens. Known positive and negative human serum titrations as well as cell controls were included in all tests.

Parainfluenza virus type 1 (HA-2 strain) was obtained from the NIH Research Resources Branch (7). Equal volumes of the undiluted CSF specimens and Eagles # 2 medium containing 50 $TCID_{50}$ units of virus were incubated 2 hr at room temperature, and then 0.2-ml volumes were added to triplicate stoppered tubes of primary Rhesus monkey kidney cells (obtained from Dr. J. A. Morris, Bureau of Biologics, FDA). After a further 2-hr incubation at 37° the tubes were fed 1 ml of Eagles #2 medium containing 2% fetal calf serum, and replaced at 37° for 3 days. The cells were then washed, 0.5 ml of a 0.5% saline suspension of fresh guinea pig erythrocytes added, and the tubes held at 4° for 2 hr. Complete absence of hemadsorption was considered to indicate the presence of antibody in the tested specimens. Known positive and negative serum and virus titrations, and cell controls were included in the test.

Results. Vaccinia antibody. None of the

127 MS specimens contained neutralizing antibody to vaccinia virus. One of the 78 control specimens contained antibody, at a titer of 1:4. This specimen came from a 58-year-old man with cerebellar atrophy.

In order to investigate the possibility that methodology was responsible for our failure to find vaccinia antibody in the CSF from MS patients, we arranged a coded exchange of specimens with Dr. Kempe's laboratory. On 15 of our negative specimens (12 MS and 3 controls), Kempe tested 14 negative and 1 (an MS specimen) positive, at a 1:4 titer. Of 20 specimens sent to us by Kempe, we found 17 positive (15 MS and 2 controls) and 3 negative (all MS). In his laboratory, 10 of these specimens (all MS) had titers ≥ 4, and 8 MS and 2 controls had titers < 4. (We had found 7 of these latter 10 specimens positive, when tested undiluted). Thus, there was excellent agreement on the absence of antibody in our own specimens, and confirmation of the presence of antibody in Kempe's specimens.

Parainfluenza antibody. None of the 62 MS or 36 control specimens of undiluted CSF contained neutralizing antibody to parainfluenza virus type 1.

Discussion. Our failure to find CSF antibody to parainfluenza virus type 1 was not unexpected, as we had already tested 65 MS and 35 control CSF specimens at a 1:10 dilution in our earlier study without finding a single positive specimen (1). However, the isolation of an agent related to this virus from 2 patients with MS obliged us to be sure we were not missing antibody in lower titer. We were not.

The presence of vaccinia antibody in Kempe's series of MS patients and its absence in our own series is intriguing. We have shown that differences in test methods are not responsible, nor is it a question of loss of antibody activity in our specimens, since we have found a significant proportion to contain antibody to measles virus, and several of these also contained antibody in at least a 1:10 titer to one or another of several other myxoviruses that were tested (1). It thus appears that the difference is real, and resides in some characteristics of the 2 populations studied. The most obvious difference is the fact that our series was entirely French whereas Kempe's was American. Could different vaccination practices in the 2 countries be involved? In France, national law requires a single vaccination at age 12, and booster vaccinations are exceedingly rare. In this country, vaccination has until recently been much more extensively practiced, but even granted the possibility of a consequently greater frequency of vaccinia antibody, its segregation, in CSF, to MS patients remains to be explained.

From the cumulated evidence in the literature and our own studies (8), we continue to believe that of all viruses suggested as possibly involved in the etiology of MS, measles virus still holds the firmest claim.

1. Brown, P., Cathala, F., Gibbs, C. J., Jr., and Gajdusek, D. C., Proc. Soc. Exp. Biol. Med. **137**, 956 (1971).

2. ter Meulen, V., Koprowski, H., Iwasaki, Y., Kackell, Y. M., and Muller, D., Lancet **2**, 1 (1972).

3. Kempe, C. H., Pediatric Res. **6**, 384 (1972).

4. Berge, T. O., and Stevens, D. A., eds., *in* "Catalogue of Viruses Rickettsiae Chlamydiae." The American Type Culture Collection, 4th ed., p. 103, Rockville, MD (1971).

5. Baron, S., Buckler, C. E., and Takemoto, K. K., Appl. Microbiol. **14**, 1042 (1966).

6. Postlethwaite, R., Virology **10**, 466 (1960).

7. Cunningham, S., and Pennington, R. M., eds., *in* "Catalog of Research Reagents," Research Resources Branch, NIAID, NIH, Bethesda, MD (1970).

8. Cathala, F., and Brown, P., J. Clin. Pathol. **25** (suppl. 6), 141 (1972).

Received Mar. 20, 1973. P.S.E.B.M., 1973, Vol. 143.