Identification of Virus-Specific Oligoclonal Bands in Subacute Sclerosing Panencephalitis by Immunofixation after Isoelectric Focusing and Peroxidase Staining

PANKAJ D. MEHTA,* BRUCE A. PATRICK, AND HALLDOR THORMAR

New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314

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We developed a sensitive peroxidase staining procedure to identify oligoclonal band specificity in subacute sclerosing panencephalitis by subjecting serum and cerebrospinal fluid to isoelectric focusing and immunofixation with measles virus. The gel was washed and stained with horseradish peroxidase-conjugated goat antihuman immunoglobulin G. The samples showed dark brown measles-specific oligoclonal bands in the alkaline pH region of the gel, whereas the controls showed no significant staining. This technique may be useful in identifying specific antibody activity against viral and other antigens in oligoclonal bands in cerebrospinal fluid and serum.

The specificity of oligoclonal bands in some inflammatory neurological disorders, e.g., subacute sclerosing panencephalitis (SSPE) and progressive rubella panencephalitis (3, 5, 8, 9, 11), is known, whereas in others, such as multiple sclerosis, the antigen causing the synthesis of oligoclonal bands has not been identified. The specificity of the bands in SSPE has been identified by absorbing serum or cerebrospinal fluid (CSF) with measles virus and eluting measles-specific immunoglobulin G (IgG) at low pH from the measles antigen-antibody complexes (5, 9). Here we report a sensitive technique for identifying virus-specific oligoclonal IgG bands in sera and CSF from SSPE patients.

Measles virus strain Edmonston, herpes simplex virus type 1, and poliovirus type 1 were propagated in monolayer cultures of Vero cells (3). Suspensions of measles virus-infected cells were divided into four equal parts. One was heated at 37°C for 30 min; the second was exposed to UV light (15-W General Electric germicidal tube) at a distance of 30 cm for 30 min with occasional stirring; the third was treated with Nonidet P-40 (final concentration, 0.1%) at 37°C for 30 min; and one was left untreated. After sonication for 1 min at 0°C, the suspensions were centrifuged, dialyzed, concentrated, and solubilized in identical volumes of phosphate-buffered saline. The herpes simplex virus- and poliovirus-infected suspensions were either heated at 56°C for 30 min or exposed to UV light. Heating completely inactivated the infectivity of both viruses, whereas some infectivity remained after UV inactivation.

Isoelectric focusing of SSPE serum and CSF was carried out as described previously (6). Briefly, 25 ml of the polyacrylamide gel containing a suitable combination of ampholines was poured between two glass plates, 115 by 230 mm (Phar- macia Fine Chemicals, Piscataway, N.J.), which were separated by a rubber gasket, and allowed to polymerize for 16 h. Focusing was carried out at 4°C at a constant current of 16 mA at 120 V and 1.5 W. These settings were monitored for approximately 1 h until the voltage reached 1,000. After 30 min at 1,000 V the focusing was completed.

For identifying measles-specific oligoclonal IgG bands, cellulose-acetate strips (10 by 50 mm) soaked with 0.1 ml of concentrated measles antigen containing 100 μg of viral proteins were applied to the gel surface and incubated at room temperature in a humid chamber for 1 h. The strips were then removed, and the gel was washed for 2 days in 0.1 M Tris-NaCl buffer (pH 7.6) to remove unbound serum proteins and excess measles antigen; 100 μl of rabbit antiserum to human IgG conjugated with horseradish peroxidase (Dako Immunoglobulins, Accurate Chemicals, Westbury, N.Y.) was then placed on the gel, and the gel was kept in a humid chamber for 1 h and then washed in the dark for 3 days in Tris-NaCl buffer. Enzyme activity was localized by immersing gels for 1 h in 3,3′-diaminobenzidine tetrahydrochloride (30 mg/100 ml; Sigma Chemical Co., St. Louis, Mo.) and 0.06% hydrogen peroxide in Tris-NaCl buffer. The enzymatic reaction was stopped by placing the gel in Tris-NaCl buffer.

The regular isoelectric focusing profiles of serum and CSF from one of five SSPE patients are shown in Fig. 1a. The oligoclonal bands seen in the pH 7.8 to 9.2 region were distinct in CSF,
whereas in serum they were somewhat masked by polyclonal IgG. This is not unusual, since measles-specific IgG content in SSPE serum is between 10 and 20% of the total IgG, compared with 40 to 60% in CSF (3). The profiles of measles-specific oligoclonal IgG bands in two of five SSPE patients are shown in Fig. 1b. Significant staining of oligoclonal bands was seen in the pH 7.8 to 9.2 region. CSF and serum from three other SSPE patients (not shown) gave similar results. Live and UV-inactivated measles virus used in immunofixation showed identical staining profiles, whereas heated and detergent-extracted virus gave somewhat weaker staining. The samples did not show oligoclonal band staining when immunofixed with concentrated preparations of herpes simplex virus, poliovirus, or Vero cells. The bands in CSF and sera from four patients with multiple sclerosis and one patient with progressive rubella panencephalitis did not stain with measles virus but showed a light-brown, diffuse background.

Our results show that measles-specific oligoclonal IgG bands can be identified in sera and CSF from SSPE patients without prior isolation of bands or absorption with measles virus and elution at low pH as previously used (5, 8, 9). Recently, Nordal et al. (7) used an imprint immunofixation method to detect virus-specific bands in SSPE and multiple sclerosis. Our method differs from theirs in using isoelectric focusing instead of agarose gel electrophoresis and antiserum conjugated with horseradish peroxidase instead of 125I-labeled antiserum and autoradiography. Although CSF applied in the gel had fourfold less IgG than the serum did, the staining intensity in both specimens was similar. The data suggest that CSF has roughly fourfold more measles-specific IgG than does serum. These findings are consistent with those reported for a quantitative method (3). In our experience, an extract of measles virus-infected Vero cells was found to be more suitable in identifying specific oligoclonal bands than was purified measles virus or hemagglutination inhibition antigen obtained from a commercial source (Microbiological Associates, Bethesda, Md.).

Recently, we (2) isolated small groups of oligoclonal IgG bands from SSPE sera with isoelectric points slightly different from one another and studied their specificity in immunoprecipitation against different measles virus antigens. Our results showed that the oligoclonal IgGs precipitated all of the measles proteins except the matrix protein.

With appropriate antigens, the method may be useful in identifying oligoclonal IgG band specificity in other chronic inflammatory disorders of humans, e.g., progressive rubella panencephalitis (11), mumps and tuberculosis meningitis (1, 10), herpes simplex virus encephalitis (7), and diseases of unknown etiology, and of animals, e.g., chronic relapsing experimental allergic encephalomyelitis (4), by employing a panel of brain antigens.

**LITERATURE CITED**


