INFECTION AND IMMUNITY, July 1982, p. 393–396
0019-9567/82/070393-04$02.00/0

Vol. 37, No. 1

Precipitation of Measles Virus Proteins by Immunoglobulin G Fractions Containing Groups of Oligoclonal Bands Isolated from Sera of Patients with Subacute Sclerosing Panencephalitis

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Received 6 January 1982/Accepted 9 March 1982

Groups of oligoclonal immunoglobulin G (IgG) bands were isolated from sera of patients with subacute sclerosing panencephalitis by employing preparative isoelectric focusing. Six IgG fractions containing two to three oligoclonal bands with different isoelectric points were used to precipitate the proteins from Vero cells infected with measles virus. The results showed that all of the measles virus proteins except the M protein were precipitated by all of the IgG fractions and that the precipitation of viral proteins by the fractions containing groups of oligoclonal IgG showed slightly different patterns in some sera, whereas other sera showed no significant differences. The present study indicates that oligoclonal IgGs in subacute sclerosing panencephalitis sera are not specific to individual measles virus proteins.

Measles virus has been implicated as the cause of subacute sclerosing panencephalitis (SSPE), a slow central nervous system disease of children. One of the important characteristics of SSPE is the presence of oligoclonal immunoglobulin G (IgG) bands in cerebrospinal fluid (CSF) and serum, demonstrable either by electrophoresis or by isoelectric focusing (IEF) (7, 9, 11). A number of studies (7, 12) have shown that the oligoclonal IgG bands represent specific measles virus antibodies. Recently, several investigators have identified antibodies against individual measles virus proteins in whole SSPE sera, using immune precipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2–5, 13). However, the antibody specificity of individual oligoclonal IgG bands has not been determined. In the present study, we isolated IgG fractions, each containing two to three oligoclonal bands, from SSPE sera and tested their specificity to individual measles virus proteins by immune precipitation.

Whole IgG was isolated by passing sera through a protein A-Sepharose 4B column as previously described (6). The IgG, when examined in immunoelectrophoresis, showed a single precipitin line with rabbit anti-whole human and anti-human IgG sera. The IgG was further fractionated by preparative IEF by the method described by Radola (10). Briefly, the gel slurry was prepared by adding 4 g of Ultrodex (LKB Instruments, Rockville, Md.) to a solution containing 6 ml of dialyzed IgG, 2.5 ml of ampholine (pH 8.0 to 9.5), 3.5 ml of ampholine (pH 9 to 11), and 88 ml of distilled water. The gel was run at 8 W constant power for 18 h at 10°C and cut into 30 fractions with an LKB fractionating grid. Each fraction was eluted with 5 ml of distilled water, and the pH was measured. They were then dialyzed, concentrated, and examined in thin-layer IEF in a polyacrylamide gel by the method described by Mehta et al. (8).

Whole IgG from the sera showed 15 to 20 distinct oligoclonal bands in a pH region of 7.5 to 9.4 (Fig. 1). In contrast to whole IgG, isolated IgG fractions showed a restricted number of bands with pIs differing from one another. The sharp distinct bands seen in the IEF pH region of 8.4 to 9.3 of whole serum IgG represent measles-specific antibodies, since most of the bands disappeared when the whole IgG preparation was absorbed with measles virus (P. D. Mehta, B. A. Patrick, and H. Thormar, Immunology, in press). In addition, we have recently identified measles-specific oligoclonal IgG bands in SSPE serum and CSF in isoelectric focusing with immunofixation with measles virus antigen, followed by staining with peroxidase-conjugated goat anti-human IgG serum. The SSPE sera showed dark-brown measles-specific bands in a pH region of 8.4 to 9.3, whereas sera from normal individuals showed no specific staining. SSPE bands were not stained by herpes simplex virus and poliovirus antigens prepared in the same manner as the measles virus antigens. Immune precipitation of

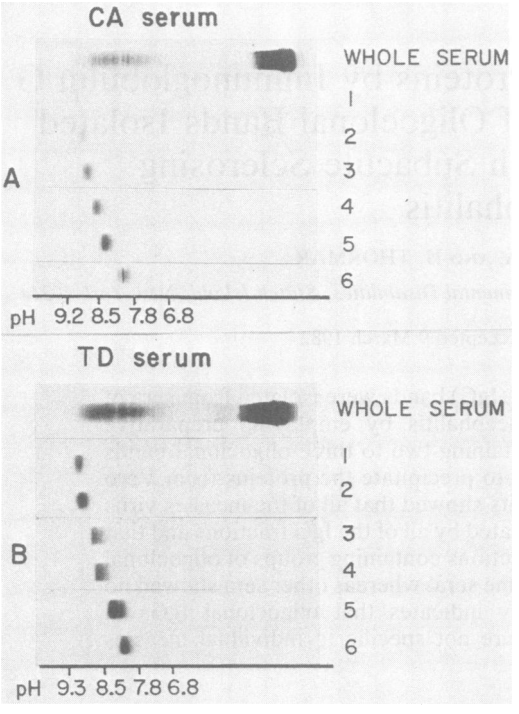

FIG. 1. IEF profiles. (A) Serum 1 whole IgG and (lanes 1 through 6) isolated oligoclonal IgG fractions with different pIs. (B) Serum 2 whole IgG and (lanes 1 through 6) isolated oligoclonal IgG fractions with different pIs.

measles virus proteins by the isolated IgG fractions was done by procedures described previously (4). We screened 12 SSPE sera with matched CSF. Figure 2 shows the protein precipitation patterns by 6 of the 12 pairs. Two of the six sera and none of the CSF had detectable antibody against the M protein. The other six pairs showed a pattern identical to that shown in Fig. 2, except only one serum contained detectable anti-M. These results confirm reports from other laboratories (2, 3, 5, 13). We selected 4 of the 12 sera for detailed study. Two of the four sera had detectable anti-M, whereas the other two did not. Fig. 3A shows immune precipitates by IgG fractions isolated from a serum that contains detectable antibody against the M protein (Fig. 2, lane 2S). This experiment showed that all IgG fractions precipitated measles virus proteins H, P, and NP. A very small amount of M protein was precipitated by IgG fractions 4, 5, and 6, which are located in the low pI regions. It was noted that the precipitation of the viral glycoprotein H was heavier in the higher pI fractions (Fig. 3A, lanes 1 and 2) than in the lower pI regions. The reverse was true for the precipitation of P. Although the data seemed to suggest some differences in the antibody specificity of IgG fractions separated by isoelectric focusing, we wished to refrain from emphasizing this conclusion. In the other three sera studied, one had detectable anti-M in the whole serum

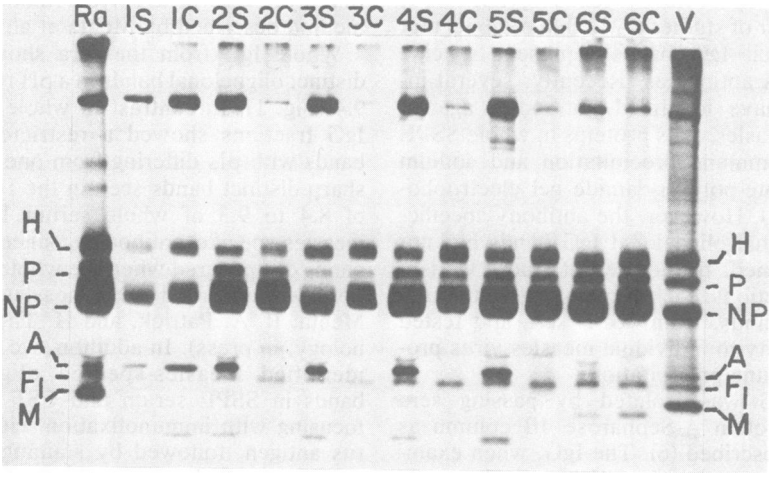

FIG. 2. Fluorograph of polyacrylamide gels containing measles virus proteins precipitated by sera and CSF from SSPE patients. The labeling of measles virus-infected Vero cells with [^{35}S]methionine, the preparation of protein extracts, immune precipitation, sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, and fluorography have been described previously (4), except 1 mM tosyl-L-lysyl chloromethane was included in the protein extracts. The reaction mixture consisted of 100 μl of protein extract and 10 or 20 μl of undiluted serum and CSF, respectively. Ra, Rabbit antiserum against Edmonston strain of measles virus; S and C, serum and CSF, respectively, of a patient; V, purified Edmonston measles virus. The nomenclature of proteins is that described by Graves et al. (1).

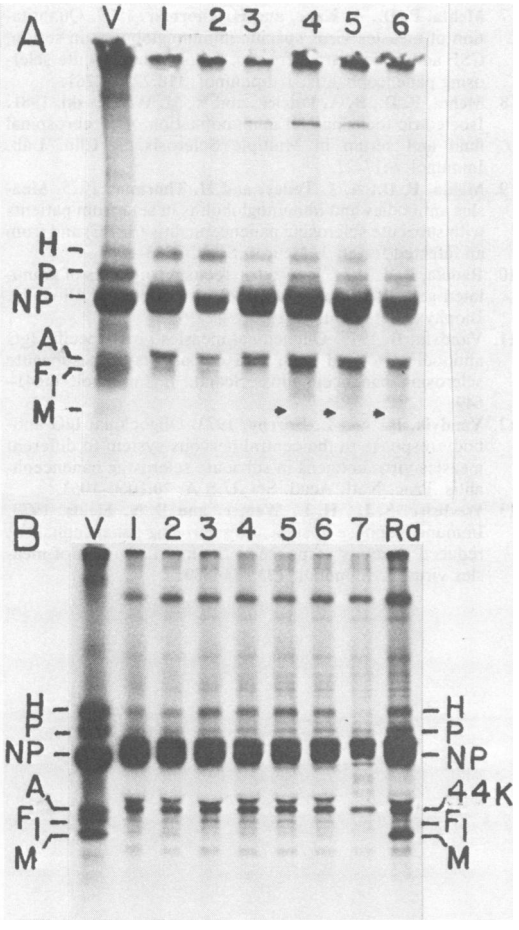

FIG. 3. Fluorographs of measles virus proteins precipitated by IgG oligoclonal fractions. All of the experimental procedures were the same as those described in the legend to Fig. 2. The approximate pI of each fraction was: lane 1, 9.3; lane 2, 9.1; lane 3, 8.9; lane 4, 8.8; lane 5, 8.6; lane 6, 8.4. (A) IgG fractions from serum 1; 350 μg per fraction. (B) Serum 2. Lane 7 in (B) was 10 μl of whole serum from patient 2. V, Purified Edmonston measles virus; Ra, rabbit anti-Edmonston (10 μl).

but not in the isolated IgG fractions and showed a protein precipitation pattern reverse to that shown in Fig. 3A. The other two, which showed no detectable anti-M, did not have any distinct pattern for serum 2 (Fig. 3B). In this serum, antibody activities against H, P, NP, and F_1 were detected in all of the IgG fractions. In contrast to Fig. 3A, antibodies against H and P were about equally distributed in every fraction. To quantitate H and P shown in Fig. 3, we cut out radioactive bands from both gels and measured the radioactivity in a scintillation counter (Table 1). The counts were in good agreement with the intensities of the bands shown in the autoradiographs.

TABLE 1. Radioactivity in protein bands[a]

Oligoclonal fraction	pI	Radioactivity (cpm) of virus proteins:	
		H	P
Serum 1			
1	9.3	2,280	1,570
2	9.1	1,970	1,220
3	8.9	1,340	1,440
4	8.8	1,850	2,360
5	8.6	1,720	2,020
Serum 2			
1	9.3	1,980	1,450
2	9.1	1,880	1,060
3	8.9	2,380	980
4	8.8	1,890	910
5	8.6	1,930	1,030
6	8.4	1,820	1,140

[a] The radioactive bands located by the exposed X-ray films were cut out from the gels and solubilized in Soluene-Liquiflour-Toluene (Packard Instrument Co.) at 60°C for 18 h, and the radioactivity was measured in a Packard scintillation counter. Results from sera of patients 1 and 2 are shown in Fig. 3A and B, respectively.

The present work indicates that oligoclonal IgG bands in SSPE sera separated according to difference in pI do not differ significantly in specificity to precipitate individual measles virus proteins. The results are in agreement with our previous studies (7), where measles antibody activities measured by different serological tests were evenly distributed in slow- and fast-moving IgG fractions isolated from SSPE sera. Because of obvious technical difficulty in isolating single individual oligoclonal bands from SSPE sera, the isolated fractions consist of two or more bands. However, the fractions represent a restricted number of bands with no detectable overlapping, as shown by immunofixation with either anti-human IgG serum or measles virus (Mehta et al., manuscript in preparation).

Our study indicates that groups of oligoclonal bands isolated from SSPE sera have antibody activity against all of the major measles virus proteins except M. The immune precipitation of measles virus proteins by the isolated IgG fractions tested in a series of experiments does not clearly identify oligoclonal IgG bands specific for any individual viral protein. However, our data suggest that, when antibody against the M protein is present in an SSPE serum, it is located in the lower pI range.

Owing to an insufficient amount of material, a similar study was not undertaken on CSF from these patients. However, when the patterns of measles-specific IgG eluted from SSPE sera and CSF were compared in IEF, striking similarities

in oligoclonal bands were seen, indicating a common origin (Mehta et al., in press).

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