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Elevated Antibody Levels Against Measles Virus P Protein in Sera of Patients with Multiple Sclerosis

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Immune precipitation followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and densitometry was used to estimate immunoglobulin G antibody levels against the H, P, and M proteins of measles virus in the sera of 24 patients with multiple sclerosis and 24 serologically matched controls. Of the 24 multiple sclerosis sera, 5 showed a statistically significant increase in antibody titer to the P protein as compared with the control sera. Antibody titers to the H and M proteins in multiple sclerosis and control sera were not significantly different.

Persistent measles virus infection causes the slowly developing, chronic neurological disease, subacute sclerosing panencephalitis (SSPE). Measles virus has also been implicated in the etiology of multiple sclerosis (MS), in large part because MS patients tend to have elevated serum hemagglutination inhibition (HI), complement fixation, and neutralization antibody titers to this virus (1, 2, 7, 8). We have previously found that sera from individuals with SSPE have reduced antibody titers against the M protein of measles virus and that these sera and sera from individuals recently recovering from natural measles infection have elevated antibody titers to the P protein of measles virus (13). Because of these findings, we were interested in determining relative immunoglobulin G antibody levels against individual measles virus proteins in sera from patients with MS.

Relative immunoglobulin G antibody levels to three measles virus proteins in the sera of 24 MS patients and 24 serologically matched sera from adults with natural measles infection in childhood were compared. A quantitative technique previously used to analyze SSPE sera was used, i.e., immune precipitation of radiolabeled measles virus proteins followed by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis and densitometry. We found that 5 of the 24 MS sera had elevated anti-P protein antibody levels. No differences were detected in antibody-levels against any other measles virus protein.

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Precipitation of [^{35}S]methionine-labeled measles virus proteins by 24 paired normal and MS sera is shown in Fig. 1. Track R shows viral proteins precipitated by hyperimmune rabbit anti-measles antiserum. Track 0 shows the control processed as in R, but without serum. The rabbit anti-measles antiserum precipitated four measles virus proteins: H, 80,000 daltons, a surface glycoprotein that is the viral hemagglutinin (9, 10, 12); P, 70,000 daltons, a phosphoprotein associated with the nucleocapsid (9, 12); NP, 60,000 daltons, the major nucleocapsid protein (9–11); and M, 37,000 daltons, the matrix protein (9). Many of the bands migrating between NP and M are breakdown products of NP (9). Recovery and detection of the remaining viral polypeptides, L (the high-molecular-weight protein) and F_1 and F_2 (the two components of the fusion protein), are highly variable (12, 13). Therefore, these proteins were not analyzed in this study. Only trace amounts of viral proteins were precipitated without serum. All 24 normal and all 24 MS sera precipitated the H and NP proteins. In contrast, precipitation of the P and M proteins was variable.

The amounts of H, P, and M measles virus proteins precipitated by the MS and normal sera were quantitated by scanning autoradiograms with a densitometer and integrating the relative areas under the peaks. Comparison of the amount of P protein precipitated by the 24 MS and the 24 normal sera by either a nonparametric sign test or Student's t test showed that there was a statistically significant increase in the amount of P protein precipitated by MS sera as compared with that precipitated by normal sera ($P < 0.01$). In addition, there was a bimodal

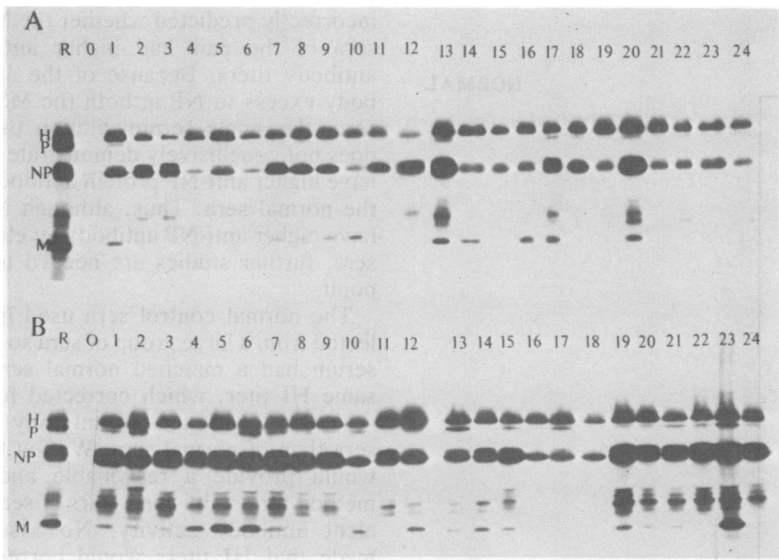

FIG. 1. Immune precipitation of [^{35}S]methionine-labeled measles virus proteins with normal and MS sera. CV-1 (African green monkey kidney) cells were infected with the Edmonston strain of measles virus, labeled with [^{35}S]methionine, and lysed with RIPA buffer (0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecylsulfate, 0.01 M Tris-hydrochloride [pH 7.2]). The resultant cell extract containing [^{35}S]methionine-labeled viral proteins was divided into 0.45-ml portions and incubated with 50 μl of test sera for 3 h at 4°C; the effective dilution of each serum was thus 1:10. Immune complexes were precipitated with protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and dissolved in 0.25 ml of gel sample buffer (5). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (5) in 10% polyacrylamide slab gels at a constant current of 40 mA for 2.5 h. Gels were dried immediately for autoradiography and exposed to film. A portion (10 μl) was applied to each gel lane. Additional details of the procedures have been described previously (12, 13). To avoid possible variations between viral protein preparations, the same cell extract preparation was used in all assays. Similar results were obtained in experiments with other preparations of [^{35}S]methionine-labeled wild-type viral proteins and SSPE proteins. Viral proteins: H, hemagglutination protein; P, nucleocapsid-associated protein; NP, major nucleocapsid protein; M, matrix protein. (A) Normal sera. These were selected from a larger group of sera so that correspondingly numbered MS and normal sera had identical measles HI titers. HI titers ranged from 1:8 to greater than 1:256. Gel lanes: R, rabbit anti-measles antiserum; 0, control (no antiserum); 1 to 24, normal sera from adults with natural exposure to measles virus in childhood. (B) MS sera. Gel lanes: R, rabbit anti-measles antiserum; 0, control (no antiserum); 1 to 24, MS sera from patients with MS.

distribution in the amount of P protein precipitated by the MS sera, whereas normal sera produced a unimodal distribution (Fig. 2). Statistical analysis of the MS sera, excluding the subgroup of 5 sera showing high anti-P protein antibody titers, revealed no significant difference between these 19 MS sera and the 24 normal sera in the amount of P protein precipitated ($P = 0.11$). The statistically significant difference in anti-P protein antibody levels between the entire population of MS sera and normal sera, therefore, was a result of the subpopulation of five MS sera that had higher anti-P protein antibody levels. The MS sera, then, consisted of two distinct populations: the main population was identical to normal sera; the smaller population had higher anti-P protein antibody levels than normal sera. Elevated anti-P protein antibody titers in the five MS sera did not correlate with HI titers or the ability to precipitate any of the other proteins analyzed. Elevated anti-P protein antibody titers also did not correlate with severity of disease, since all the MS patients in this study had equally severe cases of MS. No statistically significant differences were found in the amount of H or M proteins precipitated by the MS sera as compared with the normal sera ($P > 0.2$).

We have previously found that a valid estimate of precipitating immunoglobulin G antibody levels to the H, P, and M proteins in normal sera can be made by using a 1:10 serum dilution, as was done in the present investigation (13). However, this is not true for anti-NP antibody levels. These conclusions are based on the fact that with normal sera, there is no increase in the amount of H, P, or M protein precipitated at any serum dilutions greater than 1:10 (13), whereas apparent antibody excess is generally detected against the NP protein. We

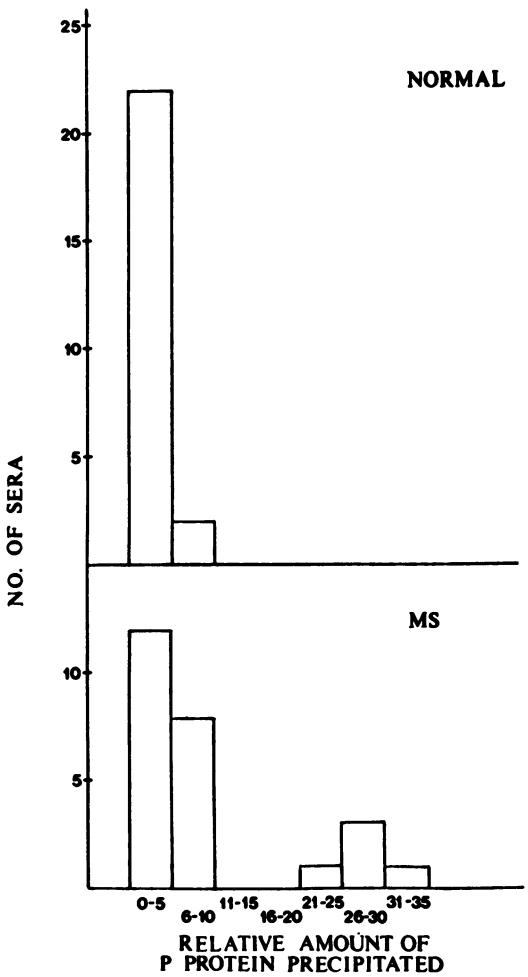

FIG. 2. Quantitation of anti-P protein antibody titers in the sera of individuals with MS. The relative amount of P protein precipitated by each serum was quantitated by scanning the autoradiograms shown in Fig. 1 and integrating the area under each peak. Numbers on the horizontal axis indicate the relative amount of P protein precipitated by the various sera in arbitrary units. The vertical bars show the number of sera that precipitated the amount of P protein in the indicated range. MS sera showed a bimodal distribution, with five sera precipitating significantly more P protein; normal sera showed a unimodal distribution.

extended these findings to MS sera by performing immune precipitation of viral proteins with twofold serial dilutions (1:20 to 1:2,560) of five representative sets of MS and normal sera (results not shown). Antibody excess was not found in our analysis of H, P, or M proteins. As expected, there was apparent antibody excess to the NP protein in all of the test sera. Maximum precipitation of NP protein occurred at serum dilutions ranging from 1:40 to 1:160. For three of the five sets of sera, the 1:10 dilution results had incorrectly predicted whether the MS or normal sera of the pair had higher anti-NP protein antibody titers. Because of the apparent antibody excess to NP in both the MS and normal sera, the single serum dilution used in Fig. 1 does not conclusively demonstrate that MS sera have higher anti-NP protein antibody levels than the normal sera. Thus, although MS sera may have higher anti-NP antibody levels than normal sera, further studies are needed to clarify this point.

The normal control sera used here were selected from a large group of sera so that each MS serum had a matched normal serum with the same HI titer, which corrected for the higher serological anti-measles antibody titers of MS sera than of normal sera. We felt that HI titers would provide a reasonable and convenient method for establishing pairs of sera with equivalent antibody activity. No assumption was made that HI titers would correspond to the ability of sera to immune precipitate the viral protein responsible for hemagglutination (protein H). In fact, we found that, although the normal and MS sera precipitated statistically similar amounts of H protein, there was only a weak, nonsignificant correlation between HI titers and the amount of H protein precipitated by each serum. Correlation coefficients for MS sera and normal sera were 0.26 and −0.07, respectively.

We have previously found that sera from individuals with the persistent measles infection SSPE and sera from individuals who have recently recovered from natural measles infection have higher anti-P protein antibody levels than sera from normal individuals (13). A comparison of the five MS sera reported here (with higher than normal anti-P protein antibody titers) with our previous results showed that the anti-P protein antibody titers in the MS sera were lower than the anti-P protein titers in SSPE sera and recent convalescent sera (results not shown). Thus, the ability of this subpopulation of MS sera to precipitate P protein fell between that of normal sera and sera from individuals with recent or persistent measles infection. The reason for the elevated anti-P protein antibody titers detected in these five MS sera is not known. They may suggest measles virus involvement in a subpopulation of MS patients. Alternatively, the elevated anti-P protein antibody titers may indicate augmented humoral immune responsiveness that is known to occur against certain viral antigens in individuals with MS (3).

A previous study of MS sera did not demonstrate any differences in the amount of measles virus proteins precipitated by MS sera as compared with control sera (4). The difference be-

tween those findings and the present findings for anti-P protein antibody titers may be due to differences in methodology (6) or sizes of patient populations.

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