Identification of Different Measles Virus-Specific Antibodies in the Serum and Cerebrospinal Fluid from Patients with Subacute Sclerosing Panencephalitis and Multiple Sclerosis1

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Matched serum and cerebrospinal fluid (CSF) samples from eight cases of subacute sclerosing panencephalitis (SSPE) and 15 cases of multiple sclerosis (MS) were characterized in neutralization, hemolysis-inhibition (HLI), hemagglutination-inhibition (HI) with Tween 80-ether-treated antigen, complement-fixation (CF), and immunodiffusion tests. CF tests were carried out with crude virus material, purified nucleocapsids, and small particle hemagglutinin as antigens. A certain diversity in the relative content of antibodies against different virus products in various sera was found. There was a high degree of correlation between titers of neutralizing and HLI antibodies, but a less strict correlation between titers of HLI and HI antibodies. Serum samples from two cases of MS and one case of SSPE contained high titers of HLI and neutralizing antibodies in the presence of only low titers of HI antibodies demonstrable with Tween 80-ether-treated antigen. The major fraction of antibodies detected in CF and immunodiffusion tests reacted with nucleocapsids. There was a tendency of nucleocapsid CF antibody titers, as compared to neutralization and HLI antibody titers, to be higher in samples from patients with SSPE than from cases of MS. No significant differences were found between antibody titers recorded in neutralization, HLI, and HI tests carried out with two different measles virus strains, Edmonston and a strain (LEC) derived from a case of SSPE. Comparison of antibodies against measles virus products and, as a reference, against a group-specific vertex capsomer antigen of adenovirus in matched serum and CSF samples revealed a production of measles virus-specific antibodies within the central nervous system of all cases of SSPE and 8 out of 15 cases of MS.

Several studies have been concerned with the quantitative occurrence of measles virus antibodies in patients with subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS). In the case of SSPE, the establishment of a prolonged virus infection in the central nervous system (CNS) is well documented by findings in both immunological and virological studies. The ratio between titers of measles virus antibodies in the serum and cerebrospinal fluid (CSF; reference 8) is less than the corresponding ratio of antibodies against a virus not related to the disease (7). This is a good indication that at least a fraction of the measles virus-specific antibodies occurring in the CSF stems from a local production of immunoglobulin G (IgG) within the CNS. Comparative analyses of measles virus-specific antibodies in patients with MS and in matched controls in most studies indicate the presence of elevated antibody titers in the former group (cf. 15, 16). This difference has not been unequivocally established by comparative testing of CSF samples (cf. 6) and there appears to be a poor correlation between antibody titers in sera and CSF samples (1, 3, 6, 18, 23). One object of the

1 The major part of this work was carried out during a visit of A. A. Salmi to the Department of Virology, Karolinska Institutet, Stockholm, Sweden.
The present study was to make an analysis of different measles virus-specific antibodies in matched serum and CSF samples from a selected group of patients with MS and for comparison with similar specimens from patients with SSPE. The titers of group-specific adenovirus antibodies in different samples were also determined. This was done in order to allow an evaluation of the relative contribution of serum antibodies to antibodies occurring in CSF.

To obtain possibly some insight into features of the prolonged infection with measles in cases of SSPE and possibly also MS, antibodies against different measles virus antigens were identified separately. This was carried out by taking advantage of experiences encountered in the study presented in the accompanying paper (12). Antibodies against nucleocapsid structures were determined in complement-fixation (CF) tests with purified antigen and in gel diffusion tests (13), whereas antibodies against envelope structures were quantified by neutralization, hemolysis-inhibition (HLI), hemagglutination-inhibition (HI) tests with Tween 80-ether-treated antigen, by CF tests with whole virus material and small particle hemagglutinin (HA) as antigens, and by gel diffusion tests (13). In certain tests, antigens derived from the Edmonston strain of measles virus as well as a strain (LEC) isolated from explanted cultures of a case of SSPE (4) were employed.

MATERIALS AND METHODS

Matched serum and CSF samples from eight cases of SSPE, plus a single serum sample from one more case of SSPE, three of which belonged to a group of patients described in a recent report (17), and from 15 well established cases of MS from the study population described in detail by Pannelius (15) were examined. Twelve of the MS patients were chosen randomly and the remaining three patients (V. M., E. N., and J. V.) were selected on the basis of detectable measles antibody in their CSF. Two of these MS patients (E. N. and J. V.) were included in a recent preliminary report on the occurrence of certain measles virus-specific antibodies in CSF and serum samples (21).

Serological techniques. The details of techniques for determination of neutralizing, hemolysis-inhibiting, hemagglutination-inhibiting, and complement-fixing antibodies were described in the accompanying investigation (12). In certain cases, both the Edmonston strain of measles virus and a strain (LEC) isolated from explant cultures of a case of SSPE (4) were used. The LEC strain was kindly made available through H. Koprowski, The Wistar Institute, Philadelphia, Pa. Cell pack antigens treated with detergents (cf. ref 12) were employed in gel precipitation assays. Details of the microtechnique used were presented previously (20). Specific rabbit hyperimmune sera against isolated nucleocapsids and small particle (10 to 14S) HA (13) were included in CF and gel precipitation tests. Group-specific adenovirus antibodies reacting with vertex capsomers were determined by penton hemagglutination-enhancement (HE) tests described in detail previously (14).

RESULTS

Correlation between antibodies determined by different serological tests. An excellent correlation was found between titers of neutralizing and HLI antibodies as illustrated by values obtained in tests of serum samples (Fig. 1). A similar correlation was found to hold true also for antibodies occurring in CSF samples. The correlation between HLI and HI antibody titers in serum (Fig. 2) and in CSF samples was found to be somewhat less strict. The presence of HI antibodies was always found to correlate with HLI antibodies of at least the corresponding titer. However, the reverse was not true. Serum samples from two MS patients and one SSPE patient contained high titers of HI antibodies in spite of the presence of only low titers of HI antibodies demonstrable by Tween 80-ether-treated antigen. CSF samples of the same patients contained HLI antibody titers at least eight times higher than HI antibody titers. Only a moderate correlation was found to exist between HLI antibody titers which, as inferred from results described above, represent a good indicator of the amount of antibodies directed against envelope structures and nucleocapsid CF antibody titers in serum (Fig. 3) and CSF samples. Samples from some patients with SSPE displayed high titers of CF antibodies against nucleocapsids in the presence of only moderate titers of HLI antibodies. In
in serum (and CSF) samples from patients with SSPE as compared to samples from cases of MS. Furthermore, the relative antibody response to nucleocapsid components as compared with HLI (or neutralizing) antibodies (Fig. 3) was higher in sera from patients with SSPE than with MS. The difference between median values of HLI and nucleocapsid CF antibodies in these groups of sera were 10 and 85, respectively. There was a trend for a similar relationship between HLI and nucleocapsid CF antibodies in CSF samples from the same patients.

The diversity of the antibody response to different measles virus components, which was observed in several cases of SSPE and also in certain cases of MS, is further elucidated by antibody titers of selected sera presented in Table 1. Examples can be found of sera (L. K.) containing high titers of nucleocapsid CF antibodies, but only moderate titers of neutralizing, HLI, and HI antibodies, or sera (R. K., J. V., A. V.) with moderate titers of neutralizing, HLI, and nucleocapsid CF antibodies, but only low titers of HI antibodies.

The serum from patient V. M. (Table 1) illustrates an exceptional pattern of divergent antibody response. This serum was found to contain moderate titers of neutralizing, HLI, and HI antibodies, but only small quantities of nucleocapsid antibodies. The poor response to nucleocapsid antigen was verified by gel precipitation assays. In all other serum samples, nucleocapsid CF antibodies were found to account for the major fraction of the overall CF antibody response (cf. Table 1).

Immunodiffusion assays confirmed the results of the tests described above. Antibodies against nucleocapsids were found in sera from all patients with SSPE and almost all patients with MS. Antibodies against envelope structures giving precipitin lines which showed identity with those produced by the reference serum against small particle HA were detectable in all sera from patients with SSPE, but only in 3 of the 15 sera from patients with MS. In addition to these reactions, the same sera from MS and SSPE patients gave one or more lines of precipitation which were different from those produced by the two kinds of reference sera. An example of such additional lines of precipitation are given in Fig. 4.

Comparative serological analysis with a strain of measles virus (Edmonston) isolated from a case of ordinary measles and a strain (LEC) isolated from a case of SSPE. Repeated comparative neutralization, HLI, and HI tests with most serum and CSF samples available for this study were carried out using antigen preparations of both the Edmonston and the LEC strain of
TABLE 1. Titers of different measles antibodies in sera from selected patients with SSPE or MS

| Patient | Disease | Titers of different measles antibodies in sera from selected patients with SSPE or MS
|---------|---------|----------------------------------------------------------------------------------------------------|
| U. H.   | SSPE   | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| L. K.   | SSPE   | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| R. K.   | SSPE   | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| R. P.   | SSPE   | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| S. L.   | MS     | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| V. M.   | MS     | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| J. V.   | MS     | Titers of different measles antibodies in sera from selected patients with SSPE or MS
| A. V.   | MS     | Titers of different measles antibodies in sera from selected patients with SSPE or MS

Abbreviations: SSPE, subacute sclerosing panencephalitis; MS, multiple sclerosis; HLI, hemolysis-inhibition; HI, hemagglutination-inhibition; CF, complement fixation.

Notes:

- Tween 80-ether-treated antigen was used.

Fig. 4. Characterization of antibodies in serum from one patient with subacute sclerosing panencephalitis (T.V.). The serum (V) diluted 1:3 (left) and 1:27 (right) and undiluted reference rabbit hyperimmune sera against purified measles nucleocapsids (N) and small particle hemagglutinin (H; reference 13) were tested against Cutscum (C) and sodium dodecyl sulfate (S)-treated concentrates of infected cells. The identity between the nucleocapsid-specific precipitate produced by the reference serum and that of patient T.V. came out clearly only at the higher dilution (1:27) of the latter. Note that in addition to the lines produced by the reference sera one extra line occurs between the serum (dilution 1:3) of patient T.V. and sodium dodecyl sulfate-treated antigen.

measles virus. These comparative analyses also included selected early and late measles convalescent sera and human gamma globulin which were used in the serological studies presented in the accompanying publication (12). In no case could a significant difference be detected between titers obtained with the two kinds of antigen preparations.

Comparison of antibody titers in sera and CSF samples. The ratio of group-specific adenovirus penton HE antibodies in matched serum and CSF samples was used as an indicator on the permeability of the membranes separating blood and CSF. In cases in which antibodies of this kind were detectable in CSF samples, a ratio value varying between 320 and 640 was found. This value is of a similar order of magnitude as that given for neutralizing antibodies against polioviruses (7).

The ratio of measles antibodies in matched serum and CSF samples from patients with SSPE was found to be reduced from the normal value in all cases studied. This was demonstrable both in neutralization (Fig. 5), HLI, and HI tests.
and in CF tests with cell extracts as antigen as exemplified by ratio values for four of the eight patients with SSPE presented in Table 2 and also in tests for nucleocapsid CF antibodies.

A different situation was found in tests of matched serum and CSF samples from cases of MS. Measles antibody titers in sera from four patients were too low to allow the calculation of a meaningful ratio. Another three patients displayed a normal ratio, i.e., one which did not differ significantly from the ratio calculated for group-specific penton HE antibodies. The remaining eight patients with MS displayed a ratio which, in one type of antibody test or another, was significantly lower than that obtained in the reference testing of penton HE antibodies (Table 2, Fig. 5). The occurrence of a low ratio was confirmed in four of these patients by results of tests for nucleocapsid CF antibodies (not shown in Table 2). Reduced ratios were more readily identified in HLI tests and in CF tests with cell extracts of measles virus-infected Vero cells. It should be mentioned that the latter test possibly can measure antibodies against both structural and nonstructural virus products.

**DISCUSSION**

Results of the present study of measles antibodies in sera and CSF samples from cases of SSPE and MS confirm certain findings made in a previous study of early and late convalescent sera from cases of ordinary measles (12). Firstly, it was found in both studies that there is a high degree of correlation between serum titers of neutralizing and HLI antibodies, but a less strict correlation between titers of HLI and HI antibodies. Occasionally, high titers of HLI antibodies may occur in the presence of only low titers of HI antibodies demonstrable by Tween 80-ether-treated antigen. This has been interpreted to indicate the occurrence of a separate hemolysin component, other than the HA, in the virus envelope. Antibodies against this hemolysin are endowed with a direct neutralizing capacity. Secondly, both studies demonstrate that, in almost all cases, antibodies against the nucleocapsid component represent the major contribution to the overall amount of antibodies demonstrable by CF and immunodiffusion tests.

There was a more pronounced diversity of the antibody response to different virus components in samples from cases of SSPE and certain cases of MS than from cases of ordinary measles. Presumably, the condition of what in the former cases might be called natural hyperimmunization can lead to a preferential antibody response towards one or more of the different virus products. It is of interest that there was a general trend of samples from patients with SSPE, as compared to those with MS, to display a relatively high titer of antibodies against nucleocapsids in relationship to titers of antibodies against other virus products (cf. Fig. 3). This could be taken to indicate the occurrence of a defective state of virus replication causing an excess production of nucleocapsids. However, an equally
plausible explanation could be that the characteristic of nucleocapsids of being a "good" immunogen becomes even more apparent under conditions of prolonged immunization.

Several studies have been devoted to comparative analyses of measles antibodies in MS patients and in different kinds of control groups. Most of these studies have shown that the average antibody titer was higher in the former than in the latter group. However, the possible occurrence of a prolonged virus infection in the CNS is difficult to evaluate, since in most studies either serum samples (cf. 2, 9, 16) or mainly CSF samples were used (cf. 6). From studies which concerned unmatched serum and CSF samples (1, 3, 6, 18, 23), it was concluded that there is no correlation between antibody levels in materials from these two sources.

It was previously shown that the ratio of serum to CSF measles antibody titers is markedly reduced in patients with SSPE (7). This was concluded from results of simultaneous testing of poliovirus neutralizing and measles virus HI antibodies. These results were confirmed by findings in the present study in which group-specific adenovirus HE antibodies were used as a reference instead of poliovirus antibodies. Low ratios were found in certain tests for measles antibodies in samples from two MS patients (21). Matched serum and CSF samples from these two patients (E. N. and J. V.) as well as 13 additional cases of MS were included in the present study. The simultaneous testing of antibodies against a nonrelated antigen (the group-specific vertex capsomer antigen of adenovirus) in the samples permitted the conclusion that 8 of the 15 MS patients had measles virus-specific IgG which had been locally produced in the CNS. The source of measles antigen inducing the production of this IgG is not known. It could be either a passage of antigen into the CNS or a virus multiplication within the CNS. In this connection it should be pointed out that a CNS involvement in connection with an ordinary measles infection may be a rather regular feature. The fact that the serum titers of measles antibodies in MS patients appear to be higher than after ordinary measles indicates that a virus infection of abnormal intensity or duration, or both, must have occurred. Millar et al. (11) have shown that sera from 4 of 43 patients with active MS contained measles virus-specific IgM. This finding suggests the possible occurrence of a prolonged virus infection. In one case, these antibodies had persisted for 3 years. Further studies of this kind are warranted. In addition, attempts should be made to identify the possible occurrence of a prolonged measles infection in cases of MS by immunofluorescent analysis of neural and extraneural tissues as well as by attempts to isolate virus from the same materials. Isolation of measles virus from lymph node biopsies of patients with SSPE was recently reported (10). Possibly it could also be of interest to follow measles virus antibodies in the CSF instead of in serum during development of the disease as was previously done (23). It should be pointed out that accentuated immunological response to viruses other than measles has been suggested in different studies to occur in patients with MS (5, 16, 19, 22). Furthermore, one study (11) demonstrated a prolonged IgM response against mumps virus in 2 of 43 patients with MS. Attempts should be made to elucidate to what extent a local production of IgG against one or more different viruses occurs in the CNS of patients with MS. However, even after this has been established, the cardinal question of whether the phenomena observed are of primary or secondary importance remains to be answered.

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LITERATURE CITED


