Comparison of Antibodies Against Different Viruses in Cerebrospinal Fluid and Serum Samples from Patients with Multiple Sclerosis

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The occurrence of a local production in the central nervous system (CNS) of antibodies against different selected viruses was analyzed by comparison of titers in serum and cerebrospinal fluid samples from groups of 50 patients with multiple sclerosis from Finland, Norway, and Sweden. Measles antibodies were determined in hemagglutination inhibition, hemolysis inhibition, and nucleocapsid complement fixation tests; mumps, parainfluenza virus type 1, and rubella virus antibodies were determined in hemagglutination inhibition tests; and herpes simplex virus type 1 antibodies were determined in passive hemagglutination tests. For reference purposes tests were also made for adenovirus antibodies in serum for hemagglutination enhancement tests and poliovirus antibodies in neutralization enhancement tests. Among the 150 multiple sclerosis patients, a local production of antibodies against measles virus was found in the CNS in 57%, against rubella virus in 19%, mumps virus in 15%, herpes simplex virus type 1 in 11%, and parainfluenza virus type 1 (Sendai) in 3%. A local production in the CNS of antibodies against any of the viruses studied was found in 71% of multiple sclerosis patients. These included 48, 16, and 7% that produced antibodies to one, two, and three or more viruses, respectively.

The occurrence of relatively higher serum titers of measles antibodies in patients with multiple sclerosis (MS) as compared with matched controls was first demonstrated by Adams and Imagawa (1). This observation was later confirmed in a number of studies, and it has further been shown that relatively increased serum titers of antibodies against other viruses may also occur (3). The frequency of occurrence of measles virus antibodies in cerebrospinal fluid (CSF) from patients with MS also is much higher than that in control patients (6). A similar observation has also been made for vaccinia antibodies (10), but a separate report could neither confirm this finding nor detect any antibodies to parainfluenza virus type 1 in CSF (5).

In further studies of the significance of increased titers of measles antibodies in MS patients and in patients with related diseases, a comparison of antibody titers in serum and CSF was made (12, 18; H. Link, E. Norrby, and J. E. Olsson, submitted for publication). The titers of measles antibodies were determined by use of three different serological techniques, and for comparison tests were also made for antibodies against viral antigens assumed not to be related to the disease. The latter tests were included to determine whether a relatively increased titer of CSF antibodies against a certain virus was due to a damage of the blood-brain barrier or to a local production of antibodies within the central nervous system (CNS). It was found that a local production of measles antibodies in the CNS occurred in about 60% of patients with MS or patients with optic neuritis or myelopathy and demonstrable oligoclonal immunoglobulin G (IgG) in concentrated CSF.

The present collaborative interscandinavian study was initiated to determine whether a local production of antibodies against viruses other than measles might occur in the CNS of patients with MS. As in previous studies, a comparison was made between the relative titers in serum and CSF of antibodies against a certain virus and the corresponding serum-to-CSF ratio of antibodies against an unrelated virus antigen. It was found that a local produc-
tion of antibodies in the CNS of MS patients against viruses other than measles may occur, although at a lower frequency.

MATERIALS AND METHODS

Study population. CSF and serum samples collected on the same day from individual patients were obtained from groups of 50 patients with MS from Finland, Norway, and Sweden. Only patients fulfilling the diagnostic criteria on MS proposed by Schumacher et al. (21) were included in the study. Some characteristics of the MS patients included in the study are summarized in Table 1. The group included a somewhat higher percentage of females than males, and the average age was about 40 to 45 years. About half of the patients had had their disease for 10 years or more, and a slightly higher proportion of the patients displayed a moderate to severe disability. About 60% of all patients had an increased concentration of IgG in CSF expressed as percentage of the total protein content. In agreement with previous findings (22), serum-to-CSF albumin ratios were lower than 100 in less than 5% of all cases, indicating the infrequent occurrence of a significant damage of the blood-brain barrier. Oligoclonal IgG could be demonstrated in concentrated CSF in all Swedish MS patients and in 44 out of 50 Norwegian MS patients. The serum gamma globulin pattern was normal in all of these patients. Electrophoretic analysis was not carried out on samples from Finland. After differential cell counting of CSF samples (Norwegian and Swedish patients only), they were centrifuged and then used for determination of the total protein concentration. The samples were stored at −20°C for less than 6 months and shipped in a frozen stage for serological testings.

Immunchemical methods. The concentrations of IgG and albumin were determined in serum and CSF samples by the single radial immunodiffusion method described by Mancini et al. (14). Specific rabbit antisera against human serum IgG and albumin were purchased from the Central Laboratory of the Netherlands Red Cross, Amsterdam. The total protein concentration of CSF was determined by a modified Lowry technique (13).

The presence or absence of oligoclonal IgG in concentrated CSF was determined as previously described in samples from Norwegian (23) and Swedish MS patients (18).

Serological tests. Tests for antibodies against measles virus, mumps virus, herpes simplex virus type 1, adenovirus (penton base-associated group reaction), and poliovirus type 1 were carried out at the Department of Virology, Karolinska Institutet, Stockholm, Sweden, and for antibodies against para-influenza virus type 1 (mouse strain) and rubella virus at the Department of Virology, University of Turku, Finland. Techniques for determination of antibodies against the following viruses were previously described: measles virus hemagglutination inhibition (HI), hemolysis inhibition, and nucleocapsid complement fixation (17); adenovirus penton hemagglutination enhancement (HE) (19); and poliovirus type 1 neutralization enhancement (NE) (18). Mumps and para-influenza virus type 1 HI antibodies were determined by conventional techniques. Samples absorbed with packed chicken erythrocytes were mixed in serial twofold dilutions with 4 U of virus hemagglutinin per dilution. After incubation for 1 h at room temperature, an equal volume of 0.5% chicken erythrocyte suspension was added, and the test was read after further incubation at room temperature. The test was carried out by use of a microtiter apparatus, and phosphate-buffered saline, pH 7.2, was used as a diluent. Rubella HI antibodies were determined by use of the technique described by Halonen et al. (7), and antibodies against herpes simplex virus type 1 were determined by use of a passive hemagglutination test performed as described in detail by Lerner et al. (11).

For practical reasons antibodies against a certain virus could not be determined in all 300 samples simultaneously. However, serum and CSF samples from individual patients were always tested in parallel, and attempts were made to include all samples from a single country in the same test. As a consequence of this arrangement, there may be certain minor variations in the sensitivity between tests for antibodies in different parts of the whole test group. The ratio of antibodies against a certain virus in serum and CSF was calculated for individual patients. If a meaningful ratio could be calculated, i.e., in cases when antibodies were demonstrable in CSF or the serum titer was equal to or higher than 160, this ratio was compared with that of antibodies against other viruses, in particular adenovirus and poliovirus, which were included primarily as references. A certain ratio was considered significantly reduced if it was four times or more lower than the corresponding ratio calculated for either or both reference antibodies.

Statistical analyses. The significance of differ-

<table>
<thead>
<tr>
<th>Study population from:</th>
<th>No. of MS patients</th>
<th>Females/males</th>
<th>Avg age (years)</th>
<th>No. of patients with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Disease for 10 years or more</td>
</tr>
<tr>
<td>Finland</td>
<td>50</td>
<td>32/18</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>Norway</td>
<td>50</td>
<td>33/17</td>
<td>45</td>
<td>32</td>
</tr>
<tr>
<td>Sweden</td>
<td>50</td>
<td>32/18</td>
<td>41</td>
<td>27</td>
</tr>
</tbody>
</table>
RESULTS

Presence of antibodies against different viruses in serum and CSF samples. Table 2 gives a summary of the lowest dilutions of sera and CSF samples examined, the frequency of occurrence of demonstrable antibodies in the samples, and in the case of sera, also the mean titer of positive samples. A comparison between the materials collected in different countries reveals the following particular features. The three different serological tests for measles virus antibodies gave somewhat varying results with samples from Finland, Norway, and Sweden, which may to some extent reflect variations in the relative sensitivity of tests carried out on different occasions. HI and hemolysis inhibition antibodies were demonstrated in a relatively lower frequency in CSF samples from Sweden. In contrast, nucleocapsid complement fixation antibodies were detected in a somewhat higher frequency in samples from this country. In mumps HI tests, a somewhat higher number of Swedish sera were positive than sera from the other countries. There was also a slight difference in mean titers of the different groups of sera. Tests for parainfluenza virus type 1 HI antibodies and herpes simplex virus type 1 antibodies gave comparable results in the three groups of samples. Both of these tests displayed a high degree of sensitivity, and with the initial dilutions used, antibodies were generally detectable in about 90% of all serum samples. Also, results of rubella HI antibody tests were similar for the three groups. Finally, as concerns reference antibodies, adenovirus HE antibody tests demonstrated the presence of detectable serum titers in all sera and in a considerable number of CSF samples, with a somewhat higher frequency in CSF samples from Finland. Some differences were apparent concerning poliovirus NE antibodies. These were detectable in a higher proportion of serum and CSF samples from Sweden than in the corresponding samples from Finland and Norway. However, the mean titers of positive sera did not differ significantly.

Occurrence of significantly reduced serum to CSF ratios of antibodies against different viruses. Adenovirus HE and poliovirus NE antibodies were included for reference purposes in the present study. Due to the presence of either one of these antibodies or both in higher titers in serum samples, a meaningful reference serum-to-CSF ratio was obtained in paired samples from 145 of the 150 patients included in the study. If a ratio of less than 140 is taken as subnormal, only 2% of patients in the whole material showed a subnormal ratio for both reference antibodies. This is to be expected since pronounced damage of the blood-brain barrier is a rather uncommon event in MS (22). In a few additional cases, either one of the two reference antibodies occurred in titers giving a

<table>
<thead>
<tr>
<th>Type of serological test</th>
<th>Lowest serum dilution tested</th>
<th>Geometric mean titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Finland</td>
<td>Norway</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>CSF</td>
</tr>
<tr>
<td>Measles virus HI</td>
<td>1:20</td>
<td>140 (50)c</td>
</tr>
<tr>
<td>Measles virus HLI</td>
<td>1:80</td>
<td>430 (50)</td>
</tr>
<tr>
<td>Measles virus nucleo-capid-CF</td>
<td>1:5</td>
<td>27 (48)</td>
</tr>
<tr>
<td>Mumps virus HI</td>
<td>1:40</td>
<td>83 (23)</td>
</tr>
<tr>
<td>Parainfluenza virus 1 HI</td>
<td>1:8</td>
<td>94 (50)</td>
</tr>
<tr>
<td>Herpes virus type 1; passive HA</td>
<td>1:40</td>
<td>930 (45)</td>
</tr>
<tr>
<td>Rubella virus HI</td>
<td>1:8</td>
<td>130 (49)</td>
</tr>
<tr>
<td>Adeno-virus HE</td>
<td>1:20</td>
<td>560 (50)</td>
</tr>
<tr>
<td>Polio-virus NE</td>
<td>1:320</td>
<td>980 (28)</td>
</tr>
</tbody>
</table>

* Abbreviations: HI, Hemagglutination inhibition; HLI, hemolysis inhibition; CF, complement fixation; HA, passive hemagglutination; HE, hemagglutination enhancement; NE, neutralization enhancement.

* A starting dilution of 1:2 was used in all tests with CSF samples, except in measles virus HLI and poliovirus NE tests, in which the lowest dilution examined was 1:4 and 1:3, respectively. Numbers indicate number of samples with demonstrable antibodies.

* Numbers in parentheses indicate number of patients with demonstrable antibodies.
subnormal ratio, but only in two and four patients were significantly reduced ratios encountered in tests for adenovirus and poliovirus antibodies, respectively (Table 3). The significance of the reduced ratio in these cases was determined from the occurrence of a more than fourfold difference from the normal ratio demonstrated for the alternative reference antibody or by comparison with ratios for antibodies against other viruses studied, e.g., parainfluenza virus type 1 or herpes simplex virus type 1.

Significantly reduced ratios of antibody titers in serum/CSF against the different viruses occurred in varying frequencies (Table 3). As concerns measles virus antibodies, reduced ratios were encountered in 42 to 66% of MS patients of different countries. The average for the whole group was 57%. The three different serological techniques used showed varying capacities to identify reduced ratios in the materials from different countries.

Rubella virus HI antibodies showed the second highest frequency of reduced ratios: 19% of patients in the whole group. There were some variations in frequencies of reduced ratios between the different countries. Only patients with a CSF antibody titer of more than 2 or a serum titer of more than 160 in the rubella HI tests were included for calculation of ratios in Table 3. This restriction was imposed because an HI antibody titer recorded with CSF samples in undiluted form or diluted 1:2 was considered to be possibly nonspecific. (CSF samples at these high concentrations could not be properly pretreated.) If, however, the test results obtained with undiluted or 1:2 dilutions of CSF samples were included, the frequency of occurrence of reduced serum-to-CSF ratios was 30, 28, and 30% in samples from Finland, Norway, and Sweden, respectively. The presence of a reduced ratio of rubella antibodies was confirmed in patients with CSF HI antibody titers of 8 or more after pretreatment of samples and in some cases also by use of the indirect immunofluorescence technique (4).

A reduced ratio of mumps virus HI and herpes simplex virus type 1 antibodies was found in 15 and 11% of patients, respectively, in the whole group. Again the frequency figures varied somewhat among the different countries. Reduced ratios of mumps virus antibodies occurred more frequently in the Finnish samples, and those of herpes simplex type 1 antibodies occurred more frequently in the Norwegian samples. A reduced ratio of parainfluenza virus type 1 HI antibodies was found in only 5 out of the 150 patients of the whole group. This was not due to a presence of low serum titers precluding a determination of a meaningful ratio, since this was definable in 62% of patients in the whole group.

A significantly reduced serum-to-CSF ratio of antibodies to more than one virus was found in some patients. The frequency of occurrence of this phenomenon was similar in samples from the three countries. A reduced ratio detectable in any of the tests used was found in 71% of all patients. In 48% of the whole group, this applied to a reduced ratio in only one test, but in 16% a reduced ratio was found in tests for antibodies against two different viruses. In 7% of the whole group, reduced ratios of antibodies against three or more viruses were encountered.

Clinical and laboratory findings in MS patients with and without a significantly reduced ratio of virus antibodies serum/CSF.

We tried to relate the occurrence of a reduced ratio of antibodies to any of the viruses studied (except those included for reference purposes) to time of onset and duration of the disease and the disability caused by the disease. In none of the samples could any significant correlations be found.

The presence of mononuclear cells in the CSF

<table>
<thead>
<tr>
<th>Source of material</th>
<th>Measles virus antibody tests</th>
<th>Mumps virus HI</th>
<th>Parainfluenza virus type 1 HI</th>
<th>Rubella virus HI</th>
<th>Herpes simplex virus type 1 passive HI</th>
<th>Adeno-virus HE</th>
<th>Polio-virus NE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All tests</td>
<td>HI</td>
<td>HLI</td>
<td>Nucleocapsid CF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>66 (96)</td>
<td>34 (72)</td>
<td>62 (96)</td>
<td>20 (24)</td>
<td>20 (30)</td>
<td>6 (40)</td>
<td>26 (74)</td>
</tr>
<tr>
<td>Norway</td>
<td>60 (98)</td>
<td>48 (66)</td>
<td>34 (98)</td>
<td>28 (30)</td>
<td>12 (30)</td>
<td>2 (62)</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Sweden</td>
<td>42 (80)</td>
<td>16 (54)</td>
<td>20 (76)</td>
<td>30 (34)</td>
<td>14 (40)</td>
<td>2 (84)</td>
<td>12 (68)</td>
</tr>
<tr>
<td>All countries</td>
<td>57 (91)</td>
<td>33 (63)</td>
<td>39 (90)</td>
<td>26 (29)</td>
<td>15 (33)</td>
<td>3 (62)</td>
<td>19 (73)</td>
</tr>
</tbody>
</table>

*Abbreviations are as in Table 2.

* Ratios were calculated only on patients with a CSF antibody titer of more than 2 or a serum titer > 160.

* Values within parenthesis give the percentage of patients with antibodies in CSF plus those with a serum/CSF ratio of > 160 in the absence of CSF antibodies.
was determined in the samples from Norway and Sweden. In the Norwegian sample, 23 patients had an increased cell count (more than 5 cells per mm³). Of these 23 patients, 18 had a reduced antibody ratio in serum/CSF to any of the viruses studied, whereas the corresponding figure was 21 out of the remaining 27 patients. In the Swedish sample, 15 out of 21 patients with an increased cell count had a reduced ratio of antibodies to any of the viruses examined, whereas only 14 out of 29 with a normal cell count in their CSF displayed a reduced ratio. The mean value of the mononuclear cell count was 6.8 (standard error of the mean [SEM] ± 1.063) in the 29 patients with reduced ratios, against 4.2 (SEM ± 0.892) in the 21 patients with normal ratios. This does not represent a significant difference.

In the samples from all three countries, a correlation was found between the occurrence of a significantly reduced ratio of serum/CSF antibodies against one or more of the viruses studied and the presence of selectively increased concentrations of IgG in CSF. In the group of 50 Swedish MS patients, the occurrence of the significantly reduced ratio to any of the viruses studied and CSF IgG expressed in percentage of total protein showed a certain significance ($P < 0.05$). The mean value of CSF IgG was 14.8 (SEM ± 0.900) among the 29 patients with reduced ratios, against 11.7 (SEM ± 1.054) among the 21 patients with normal ratios ($P < 0.05$). Similarly, in the Finnish group the mean IgG concentration in CSF of patients with a reduced ratio of measles HI antibodies differed significantly from those without a reduced ratio ($P < 0.001$). A similar difference of somewhat lower degree of significance ($P < 0.05$) was found for mumps virus HI antibodies.

The relationship of presence or absence of oligoclonal IgG in concentrated CSF to the occurrence of a reduced ratio of antibodies in serum/CSF was analyzed in the Norwegian group. Out of the 44 patients with demonstrable oligoclonal IgG, 35 displayed a reduced ratio of antibodies. However, among the six patients who did not have detectable oligoclonal IgG, three had reduced ratios. In two cases a reduced ratio was demonstrated in tests for herpes antibodies and in one test for measles antibodies.

**DISCUSSION**

The relationship between measles virus antibodies in serum/CSF of patients with MS and related diseases has been studied previously (12, 18; Link et al., submitted for publication). It was found that in 50 to 60% of patients with MS and optic neuritis or myelopathy combined with a presence of oligoclonal IgG in CSF, a reduced ratio of measles antibodies in serum/CSF could be detected. There was a correlation between the occurrence of selectively increased IgG in CSF and the presence of a reduced ratio. The occurrence of a reduced ratio was concluded to reflect a local production of measles virus antibodies in the CNS. This in turn was interpreted to indicate a presence of measles virus antigen in the CNS of MS patients during some period after their acute measles disease and preceding or concurrent with their MS disease.

This study has shown that a significantly reduced ratio of virus antibodies in serum/CSF may concern not only those directed against measles virus antigens but also against certain other viruses. In agreement with previous findings (18), our study showed a local production of measles virus antibodies in the CNS in 40 to 60% of all MS patients. Measles virus antibodies were analyzed by use of three different serological techniques, whereas only single techniques were used to study antibodies against other viruses. This should be considered when frequencies of reduced ratios of antibodies to different viruses are compared. Besides antibodies to measles, antibodies to rubella virus, mumps virus, and to some extent herpes simplex virus type 1 were found to be locally produced in the CNS of a certain fraction of MS patients. The frequency of occurrence of this local production of virus antibodies varied between 19 and 11% in the whole material. Only in few cases could a local production in CNS of parainfluenza 1 virus HI antibodies be detected. It should be mentioned that the parainfluenza virus type 1 strain used in our serological tests was of murine origin and therefore partly different from the strain of parainfluenza virus type 1 isolated from two patients with MS (15).

The proposed presence of measles virus antigen in the brain of patients with MS mentioned above has been suggested to be due to an activation of latent measles virus infections in the CNS. In analogy with this, it is possible that the local production of antibodies in the CNS to other viruses also may be due to activation of latent infections. The differences as concern the frequency of occurrence of such a local production of antibodies to a certain virus in the CNS of MS patients may reflect varying tendencies for establishment of a state of latency or different conditions of latency for different viruses. It can be inferred from the present findings that
the hypothesized activation of latent infection may concern more than one type of virus.

An alternative explanation for the present finding might be a nonspecific activation (without the presence of any antigen) of immune competent cells conditioned to produce antibodies against different viral antigens. However, against this speaks the recent preliminary observation (B. Vandvik and E. Norrby, manuscript in preparation) that antiviral antibodies at least in some cases represent a certain fraction of the oligoclonal IgG produced within the CNS of MS patients. Oligoclonal IgG in concentrated CSF has been absorbed onto purified measles virus antigen and then eluted at a reduced pH. Similar studies could be carried out with MS patients in whom a local production of antibodies against more than one virus can be demonstrated. In such a study, purified antigens of each of the viruses concerned could be used for absorption and elution of antibodies. Possibly oligoclonal IgG of different electrophoretic mobility might be absorbed to different virus antigens. It appears likely that the development of an oligoclonal IgG response, in cases when one is not dealing with IgG-producing tumor cells of myeloma character, should be based on a selection by antigen of cells producing antibodies of high avidity.

In the Norwegian material two patients without demonstrable oligoclonal IgG in concentrated CSF displayed a local production in CNS of antibodies to herpes simplex virus type 1. This is not due to any lack of capacity of this virus to evoke a production of specific oligoclonal IgG, since this was recently identified in concentrated CSF of cases of herpes encephalitis (Vandvik and Norrby, in preparation).

An increased frequency of occurrence of HL-A3 and HL-A7 antigens in MS patients has been demonstrated (2, 8). In a separate study (9), this correlation was further examined by use of a mixed-lymphocyte culture technique. A strong correlation was found between a specific lymphocyte determinant, LD-7a, probably linked to the HL-A7 antigen and progressive MS. A genetic condition reflected by the occurrence of certain lymphocyte determinants may predispose certain individuals to react in an abnormal fashion to infections with certain viruses. This abnormal reaction might imply an increased tendency to (i) establish latent virus infections, (ii) activate such infections, or (iii) react in an abnormal immunopathological way in connection with a possible activation of latent infections. It is of considerable interest that in a recent report (16), patients contracting paralytic polio had HL-A3 and HL-A7 antigens in a significantly higher frequency than individuals who did not display signs of CNS involvement when infected by the virus. A further analysis of the possible importance of genetic factors in the MS disease preferably should include serological studies on the occurrence of antibodies against viral antigens.

ACKNOWLEDGMENTS

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LITERATURE CITED


