
Intrathecal Synthesis of Virus Antibodies in Multiple Sclerosis Patients

THORGGERDUR ARNADOTTIR, MAURI REUNANEN, and AIMO SALMI

Department of Virology, University of Turku, 20520 Turku 52, and Department of Neurology, University of Oulu, Oulu, Finland

Received 5 May 1982/Accepted 13 July 1982

A follow-up study on the intrathecal synthesis of viral antibodies in multiple sclerosis patients was made on 28 patients over a period of about 2 years. Serial serum and cerebrospinal fluid specimens were assayed for antibodies against measles, rubella, parainfluenza type 2, respiratory syncytial, mumps, influenza A, influenza B, adeno, and herpes simplex viruses by employing a solid-phase enzyme immunoassay technique. All patients had local antibody synthesis against one or more of the antigens studied. Rubella and measles virus antibodies were found with the highest frequency and were synthesized at the highest rate. Simultaneous intrathecal antibody synthesis against the greater number of the viruses studied was associated with higher local immunoglobulin G synthesis. A good overall correspondence in the fluctuations of the different viral antibodies synthesized intrathecally was usually found. Sometimes the changes in intrathecal antibody levels correlated well with the changes in immunoglobulin G index and sometimes not. These fluctuations could not be correlated with the clinical course of the disease. The results of this study suggest that the viral antibodies studied are not relevant to the etiology or the pathogenesis of multiple sclerosis.

Multiple sclerosis (MS) is a chronic relapsing and remitting neurological disease characterized by scattered lesions of myelin loss in the white matter of the central nervous system (CNS). Its cause is unknown, but epidemiological and immunological evidence tends to implicate a viral agent (21). Immunopathological phenomena are hallmarks of this disease (2), which suggests that autoimmunity is important for its pathogenesis.

Most MS patients synthesize immunoglobulin G (IgG) intrathecally (7, 15). The antibody specificity of this IgG is still largely unknown. Viral antibodies studied so far seem to account for only a fraction of the bulk of the locally produced IgG (18, 34, 35). One approach for studying the role of intrathecal antibody synthesis in the etiology and pathogenesis of MS is to examine changes in individual patients through different times and phases of the disease. Changes in synthesis rate related to the clinical course of the disease would suggest a specific role for those antibodies and corresponding antigens in the pathogenesis of MS. Few studies of this kind have been carried out, and these have not so far given clear results (1, 21, 23, 27).

We report here a follow-up study on 28 patients with MS. Serial serum and cerebrospinal fluid (CSF) specimens were assayed for antibodies against nine different virus antigens to estimate the degree and fluctuation of intrathecal antibody synthesis. The results were compared with intrathecal IgG synthesis and clinical parameters.

MATERIALS AND METHODS

Patients. A total of 28 patients with confirmed MS (11 male and 17 female) were included in this study. The diagnosis was based on generally accepted criteria as reported by Schumacher et al. (31). This study was undertaken with the patients' consent, and they were monitored for about 2 years (1977 to 1979) by one of us (M.R.). The course of the disease and details of treatment were recorded during this period. The neurological deficit value was assessed by Fog's scale (5).

Four of the patients were in the stationary phase throughout the study period, three were classified as chronic progressive, and twenty-one had a typical relapsing-remitting course of disease. At the end of the follow-up period, 5 to 15 serum-CSF specimen pairs were available per patient, totaling 217 serum-CSF specimen pairs. Specimens taken when patients were suffering a deterioration of the disease with new clinical symptoms and signs before the onset of corticosteroid treatment were chosen to represent exacerbations, and specimens taken at least 3 weeks after onset of the foregoing remission represented remissions. Serial serum specimens from 11 healthy individuals (7 male and 4 female), 5 to 47 specimens from each (totaling 145 specimens), were tested for comparison. All specimens were stored at -40°C until tested.

IgG and albumin assays. The concentrations of IgG and albumin in the serum and CSF specimens were determined by the single radial immunodiffusion meth-
TABLE 1. Patients with elevated average antibody indexes for one or more of the viruses studied and the median IgG index of each group

<table>
<thead>
<tr>
<th>No. of elevated avg antibody indexes</th>
<th>No. of patients</th>
<th>Mean IgG index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1.53</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.89</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.94</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>2.36</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1.81</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2.34</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>2.11</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2.04</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0.68</td>
</tr>
</tbody>
</table>

od described by Mancini et al. (16). From these results, the CSF/serum ratio for IgG and albumin and the IgG index, [IgG(CSF) × albumin(ser)/IgG(ser) × albumin (CSF)], for every serum CSF pair were calculated (3, 8, 25). Values exceeding 0.60 in the IgG index were taken to indicate intrathecal synthesis (32). The CSF/serum ratio of albumin was used as an indicator of blood-brain barrier (BBB) integrity, and a ratio of 0.0075 was used as the upper normal limit (25, 32).

Virus antibody assay. Solid-phase enzyme immunoassay was employed to measure antibodies against measles virus (MV), herpes simplex virus (HSV), respiratory syncytial virus (RSV), mumps, parainfluenza virus type 2 (parainfl-2), influenza A virus, influenza B virus, rubella virus, and adenovirus. The preparation of the antigens has been described earlier for the following: semipurified rubella virus (20), purified hexon antigen of adenovirus type 2 (9, 26), and virus-infected cell lysates of RSV, MV, mumps (36, 38), influenza A, influenza B, parainfl-2 (19), and HSV (13). The antigens were diluted in phosphate-buffered saline, pH 7.4 (PBS), and used to coat the solid phase in the following protein concentrations: rubella, 3 µg/ml; adenovirus, 20 µg/ml; MV and RSV, 10 µg/ml; mumps, influenza A, and influenza B viruses, 20 µg/ml; parainfl-2, 10 µg/ml; and HSV, 25 µg/ml.

Polystyrene flat-bottomed microtiter plates (no. 76-201-05, Flow Laboratories) were used for the solid phase. They were coated with 0.1 ml of antigen solution per well overnight at 4°C. After being washed with PBS containing 0.1% Tween 20, they were incubated with specimen dilution buffer (PBS, 0.5% bovine serum albumin, 0.5% Tween 20) for 15 min at 37°C. The plates were then emptied and used damp. Specimen dilutions were made in 0.1-ml volumes for each well to the plates and incubated at 4°C overnight, after which the plates were washed twice with washing solution (0.5 M NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 6.5 mM Na₂HPO₄, 2H₂O, 0.05% Tween 20, 0.1 mM Thiomersal LDBH Chemicals Ltd.) in distilled and deionized water). Rabbit antibodies against human IgG (DAKO, lot 110C, code A090), diluted 1/40,000 with specimen dilution buffer, were added in 0.1-ml volumes per well, and the plates were incubated for 1 h at 37°C. The plates were then washed twice as described above. Horseradish peroxidase-conjugated swine antibodies against rabbit IgG (Orion Diagnostica) diluted 1/2,500 in PBS-2% Tween 20–20% inactivated swine serum and 0.1 mM Thiomersal were added in 0.1-ml volumes per well and incubated a further 1 h at 37°C. After two washing cycles as above, 0.15 ml of the substrate solution consisting of 3 mg of orthophenylene diamine per ml (Koch-Light Laboratories) in 0.1 M citrate–Na₂HPO₄ buffer, pH 5.5, and 0.01 ml of 30% H₂O₂ per 15 ml of buffer was added. After incubation for 1 h at room temperature in the dark, the reaction was stopped by adding 0.15 ml of 1 M HCl. The intensity of the color at 492 nm was measured with a Titertek Multiscan photometer (Ehlab).

The starting dilutions of the serum specimens varied from 1/200 to 1/600, and those of the CSF specimens were from 1/5 to 1/20, depending on preliminary tests. Seven twofold dilution steps were made on serum, and five were made on CSF specimens. A titration curve was drawn on each sample, and its linear part was compared with the linear part of a twofold dilution curve of a standard serum pool (positive reference curve). The relative antibody titers were estimated by measuring the distance as the log, from the positive reference curve (1, 12). The corresponding serum and CSF specimens were always tested on the same plate, and the positive reference was included on every plate. All specimens from each patient or control subject were tested at the same time.

The results were expressed as the antibody index, which, according to the IgG index calculations, was defined as the CSF/serum antibody ratio divided by the CSF/serum albumin ratio. To determine the upper normal limit of the antibody index, we calculated antibody indexes for MV and RSV antibodies for 28 patients with other neurological diseases (37). The mean plus two standard deviations were 0.49 + 0.32 = 0.81 for MV and 0.41 + 0.32 = 0.73 for RSV. Therefore, antibody indexes exceeding 0.80 were taken to indicate intrathecal antibody synthesis.

Statistics. Spearman’s rank correlation test was used to compare the IgG index and the number of elevated average antibody indexes. Fischer’s exact probability test was used for comparison of changes in neurological deficit and changes in antibody indexes during the follow-up.

RESULTS

Intrathecal antibody synthesis in the MS patients. The average values of the IgG indexes of the patients ranged from 0.7 to 3.5. This indicated that all patients were synthesizing IgG in their CNS (32). The CSF/serum albumin ratios showed that two patients had moderate damage in BBB with average CSF/serum albumin ratios of 0.0076 and 0.0086, and one had greater BBB damage with an average CSF/serum albumin ratio of 0.0130. One patient showed transient BBB damage.

Because many CSF-serum specimen pairs were tested per patient, every patient had many antibody index values for each antigen. The average antibody index for each antigen was the basis for assessing the intrathecal antibody synthesis. All patients had intrathecal antibody synthesis against one or more of the nine viruses studied (Table 1). Rubella virus antibodies were
found with the highest frequency and at the highest levels, closely followed by MV antibodies (Table 2 and Fig. 1). Those patients who had intrathecal antibody synthesis against a majority of viruses usually had higher IgG indexes than those with local antibody synthesis against only a few of the viruses studied \( (P < 0.01, \text{Spearman's rank correlation test}) \) (Table 1). In some patients, the intrathecal synthesis of antibodies against one or more viruses far exceeded the antibody production against the other viruses (Fig. 2).

**Virus antibody synthesis during follow-up.** The serum virus antibody titers were generally rather stable during the follow-up period both in the MS patients and the healthy control group (Table 3). Relatively great titer changes were occasionally observed in both groups (Table 3). The fluctuations were of similar magnitude for all nine virus antibodies studied. The MS patients showed slightly greater instability in serum virus antibody titers than the control subjects (Table 3). As there were no CSF specimens available from the control group, the follow-up study of intrathecal virus antibody synthesis was only made on the MS group. On the average, the change in antibody index to each virus from lowest to highest value was slightly less than twofold in all of the virus tests (Fig. 1). Fourteen patients showed threefold or greater changes in antibody indexes to one or more viruses, with no particular virus being favored (Fig. 3 to 5). Two patients had less than a twofold change in their antibody indexes to all nine viruses (Fig. 6). The changes in IgG index observed in the patient group during the study period were twofold or less.

![Image](http://iai.asm.org/)

**FIG. 1.** Intrathecal antibody synthesis and changes in antibody indexes during follow-up of the MS patients. (A) Average antibody index for each virus. (B) Change in antibody index calculated as maximum index divided by minimum index for each patient during follow-up.

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. (% of patients with elevated antibody index</th>
<th>No. of times each antibody type represented some patient's highest avg antibody index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubella</td>
<td>26 (93)</td>
<td>13</td>
</tr>
<tr>
<td>MV</td>
<td>24 (86)</td>
<td>5</td>
</tr>
<tr>
<td>Parainfl-2</td>
<td>18 (64)</td>
<td>2</td>
</tr>
<tr>
<td>RSV</td>
<td>16 (57)</td>
<td>4</td>
</tr>
<tr>
<td>Influenza A</td>
<td>15 (54)</td>
<td>2</td>
</tr>
<tr>
<td>Influenza B</td>
<td>14 (50)</td>
<td>0</td>
</tr>
<tr>
<td>Mumps</td>
<td>11 (39)</td>
<td>1</td>
</tr>
<tr>
<td>Adeno</td>
<td>8 (28)</td>
<td>0</td>
</tr>
<tr>
<td>HSV</td>
<td>6 (21)</td>
<td>1</td>
</tr>
</tbody>
</table>
their fluctuations (Fig. 7). Occasionally, the levels of antibodies to one virus showed different changes from the other virus antibodies (e.g., parainfl-2 in Fig. 3), but these changes were not limited to any single virus. The changes in antibody indexes sometimes showed a good correlation with the changes in overall intrathecal IgG synthesis and sometimes not (Fig. 4, 5, and 7).

Relation of intrathecal antibody synthesis to clinical findings. The intrathecal antibody synthesis during exacerbations and remissions of the disease was studied by calculating and comparing the average antibody index of specimens taken during exacerbations and remissions in the same patient. This comparison was possible for 18 patients. Three patients had an average antibody index of specimens taken during exacerbations which was higher than the average antibody index of remission specimens for all of the antibodies studied. One patient had for all of the antibodies studied an average antibody index of remissions that was higher than the average index of specimens taken during exacerbations (Fig. 4). The majority of the patients had antibody indexes to some viruses higher in exacerbations than in remissions, and for indexes to other viruses the opposite was true.

When the levels of virus antibody indexes were monitored in each patient, fluctuations occurred both at the time of exacerbations (Fig. 4 and 5) and also during long periods of remission (Fig. 4 and 7). Exacerbations of the disease could also occur without any major change in antibody indexes (Fig. 6). The changes in antibody indexes in the three patients with a chronic progressive course of the disease during the follow-up did not differ clearly from those of the patients with a relapsing and remitting disease course. The four patients with a stationary course of disease had relatively stable levels of intrathecal synthesis; only in one instance did the antibody index show more than a threefold change. However, the difference in the size of the patient groups made more exact comparison difficult.

The changes in antibody indexes sometimes showed a good relationship to the changes in neurological deficit (5), and sometimes they did not (Fig. 5 and 7). Although the changes in antibody index levels could not be related to the clinical course in individual patients, it seems that on the whole the patients with more active disease (greater changes in neurological deficit) had more changes in their intrathecal antibody synthesis. The five patients who showed over 10% change in neurological deficit had a significantly higher frequency of threefold or greater changes in antibody index than the patients with more stable disease ($P < 0.01$, Fischer’s exact probability test).
TABLE 3. Changes in relative serum antibody titer during follow-up on MS patients and healthy control subjects

<table>
<thead>
<tr>
<th>Virus</th>
<th>Smallest</th>
<th>Largest</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>Control</td>
<td>MS</td>
</tr>
<tr>
<td>Rubella</td>
<td>0.0</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>MV</td>
<td>0.4</td>
<td>0.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Parainfl-2</td>
<td>0.2</td>
<td>0.2</td>
<td>2.5</td>
</tr>
<tr>
<td>RSV</td>
<td>0.2</td>
<td>0.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Influenza A</td>
<td>0.2</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Influenza B</td>
<td>0.2</td>
<td>0.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Mumps</td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Adeno</td>
<td>0.3</td>
<td>0.2</td>
<td>2.6</td>
</tr>
<tr>
<td>HSV</td>
<td>0.2</td>
<td>0.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

* The relative antibody titers were estimated by comparing the titration curve of the serum to the positive reference curve. The change is defined as the maximum relative titer minus the minimum relative titer observed in each individual.

DISCUSSION

The continuous intrathecal production of various viral antibodies in multiple sclerosis observed in the present study confirms earlier findings (1, 21, 27, 33). All of the 28 patients in this study had local production of antibodies in the CNS against one or more of the nine viruses tested. No specific fluctuations related to clinical course were found, suggesting that these antibodies are not relevant to the pathogenesis of MS.

FIG. 3. Follow-up of one MS patient (male, born 1947, MS onset 1971) showing the course of the antibody indexes, IgG index, and neurological deficit (%) (symbols in A and B are as for Fig. 2). This patient had a chronic progressive course during the study period and did not receive corticotropic treatment. The maximum CSF/serum albumin ratio was 0.0041 (December 1977), indicating an intact BBB throughout the observation period.
Intrathecal antibody synthesis against rubella and MV antigens occurred most often, which is in agreement with earlier studies (6, 22, 29). After these, parainfl-2 and RSV antibodies were most frequently produced. Thus, we failed to confirm the observation of Vartdal et al. (35) that viruses with neurotrophic properties tend to be more frequently associated with local synthesis in the CNS in MS. HSV antibodies were synthesized at the lowest rate of the nine viral antibody types studied. This comparatively low frequency is in agreement with some earlier studies (22), but a higher incidence of locally produced HSV antibodies has also been reported (6, 28). All but one patient had intrathecal synthesis against some of the paramyxo-type viruses studied (MV, parainfl-2, RSV, or mumps), and all but two had intrathecal synthesis against rubella plus some of these viruses. This finding could imply a role for these viruses in the etiology of MS, but immunological hyperactivity may be a more likely explanation as fundamental defects in immunoregulation are probably present in MS (2). MV and rubella antibodies have been shown to be elevated in peripheral blood in other diseases where immune functions are disturbed, such as systemic lupus erythematosus and chronic active hepatitis (14).

Both the IgG indexes and the antibody index-
es calculated in this study were fluctuating during the observation period in these patients. The different antibody indexes were usually fluctuating in rather good accordance in individual patients, which agrees well with our earlier study on MV, rubella, and RSV antibodies (1). The fluctuations in antibody indexes in the present study sometimes correlated well with the changes in the IgG index and sometimes not. Both features could be observed in one and the same patient. This could partly be due to technical reasons, but this phenomenon may also reflect some true immunological processes in the CNS. The bulk of the intrathecal immunoglobulin synthesis may be under control of a local fluctuating regulatory mechanism, which could lead to general changes in IgG synthesis. Since specific antibodies seem to represent only a minor part of the total immunoglobulins (18, 34, 35), some antibodies may not necessarily follow the general upward and downward trends of the CSF immunoglobulin levels, especially if the synthesis of the different antibody specificities is not under similar regulatory control. It has been demonstrated that immunoglobulins extracted from different parts of the brain are qualitatively different (17). Since disease activity may not necessarily be in the same immunological phase in all parts of the brain, some discrepancies in the synthesis rates of different antibodies are not a very unexpected finding.

The changes in antibody indexes during the follow-up did not relate well to the clinical findings as had been noticed earlier in MS (1, 4, 21, 38). However, the four patients with a stationary phase of the disease did have relatively stable antibody indexes, and patients showing great changes in neurological deficit during the follow-up tended to have relatively large fluctuations in antibody indexes. These observations suggest that patients with a more malignant course of disease may have a more labile control of immunoglobulin synthesis in the CNS. Similar, CSF immunoglobulin abnormalities have been found more often and have been more pronounced in the malignant course of the disease (24).

If intrathecal antibody synthesis against only one particular virus increases during the course of MS, it may indicate that the corresponding viral antigen is being expressed at that time locally in the brain. Such changes were observed in some of these patients. One patient showed a large increase in his already high levels of parainfl-2 antibodies in his last three specimens (Fig. 3). Another patient (Fig. 6) did not have an elevated antibody index against RSV at the beginning of the study period. After this pa-
tient’s second exacerbation, antibody indexes against RSV started to increase and had reached abnormal levels in the last three specimens. Although it cannot be excluded that parainfl-2 and RSV antigens were expressed in the brains of these two patients, some other explanations for these changes may be as likely.

Studies on MS patients earlier vaccinated with bacterial toxoids have suggested that B cells committed to these antigens outside the CNS may later on enter the CNS and produce antibodies (30). It is not known, however, how common the introduction of new plasma cells into CNS is during ongoing MS. The changes in antibody levels among these patients may well reflect such relocations of B cells when the BBB is temporarily disturbed (10). This may be a valid explanation, at least for the latter patient (Fig. 6), because the CSF/serum albumin ratio reached 0.0070 in the second exacerbation, which is very close to the upper normal limit for the BBB integrity, whereas at other times it was about 0.0055. As mentioned above, antibodies in various parts of the brain may have different specificities (17). Therefore, an increase in certain antibody specificities in CSF at different times may also occur after activation of different clones in different locations of the brain.

Antibody changes occurred in exacerbations and also during long remission periods, which might reflect clinically silent relapses (11). Comparison of the antibody indexes during relapse to those during remission did not give any uniform results. In fact, this is not surprising, since it was found that during one exacerbation different changes could occur within a short period of time. Hence, a part of the immunological changes might escape detection.

The pathogenetic role of local synthesis of viral antibodies in MS is still unexplained. It now seems clear that changes in intrathecal synthesis can occur in a short time. Many changes may therefore escape detection even if carefully planned longitudinal studies. It is our feeling that clinical changes and immunological changes could act as separate entities. More immunological changes in patients with more active disease may be a reflection of the greater defects in immunoregulation and more nervous tissue damage. The lack of concordance between the immunological course and viral antibody synthesis, at least for the viruses studied here, tends to diminish their role in the etiology and the pathogenesis of MS.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council, the Academy of Finland, and the Sigrid Juselius Foundation.

The skilful technical assistance of Erja Laakso is gratefully acknowledged.

LITERATURE CITED


Downloaded from http://iai.asm.org/ on January 11, 2018 by guest


