Cytotoxic Antibody to Cells Infected with Measles Virus in Serum and Cerebrospinal Fluid of Multiple Sclerosis and Control Patients

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Sera and cerebrospinal fluids (CSFs) from 66 patients selected from a larger sample of multiple sclerosis (MS) and control patients were studied for presence of complement-dependent cytotoxic (CT) antibody against baby hamster kidney cells infected with measles virus, strain Lec. The MS group contained 26 patients with clinically definite disease and 7 with probable MS. Seventeen of the 33 patients selected from the MS group had hemagglutination-inhibiting (HI) antibody to measles virus in their CSFs. Specimens from 33 control patients with other identifiable neurological disorders were matched according to the time of specimen sampling and with the age of the donors. Seven of the controls had HI CSF antibody. The serum CT geometric mean antibody titer of the MS group was approximately twofold higher than that of the control group. Forty-two percent of the MS group and 18% of the control group had CT antibody in the CSF. With the exception of the ratio of one control patient, the serum/CSF ratios of CT antibody from all patients were 128 or less. Nine CSFs (six MS and three control specimens) had CT antibody but no detectable HI antibody. Conversely, 12 CSFs (eight MS and four control specimens) had HI antibody but no detectable CT antibody. Five patients in the MS group with both kinds of CSF antibodies had reduced CT ratios but normal HI ratios. The results suggest that the two tests detect CSF antibodies reactive with different antigens. In this study, where less than half of the MS patients displayed CSF CT antibody, it is unlikely that such antibodies play an active role in the pathogenetic mechanism operative in the disease.

Despite extensive study, the etiology and pathogenesis of multiple sclerosis (MS) remain unknown. The presence of antibody to measles virus in the cerebrospinal fluid (CSF) of a high percentage of MS patients (1) led to the theory that measles virus infection of the central nervous system may be the primary cause of the disease or may play a contributing role by inducing an autoimmune response to altered or shared cellular membrane antigens (6). Cytotoxic antibody or cytotoxic killer cells elicited by virus-induced membrane changes in infected cells of the central nervous system could, through their interaction with membrane antigens, contribute to the pathogenesis of the disease.

This study presents data on the presence of complement-dependent cytotoxic (CT) antibodies in the serum and CSF of MS patients and control patients with other neurological diseases.

MATERIALS AND METHODS

Patient selection. As previously described (8), serum and CSF were obtained from patients who gave informed consent to be studied and who were clinically characterized over a 2- to 3-year period by neurologists using the criteria of McDonald (15). Patients with identifiable neurological diseases other than MS, matched as to age and time of serum and CSF sampling with the MS group, served as controls (Table 1). Sixty-six patients from a group of 298 patients, who were studied by a variety of measurements, the results of which will appear elsewhere, were selected for determination of CT antibodies. Within the entire patient pool, 21% (28/133) of the MS group and 7.8% (13/165) of the control group had hemagglutination-inhibiting (HI) antibody to measles virus in their CSFs. Seventeen of these CSFs from the MS group and seven of the CSFs from the control group were in sufficient quantity for study by the cytotoxicity test. The number was brought to 33 in each group by the addition of 16 CSFs lacking CSF HI antibody from the MS group and 26 CSFs from the control group. The

average age in the MS group was 43 years, and in the control group, 46 years.

Assay for CT antibody. The assay for CT antibody was performed as previously described in detail (5), using a baby hamster kidney cell line (BHK 0-853) infected with the Lec strain of subacute sclerosing panencephalitis measles virus (2). Briefly, doubling dilutions of serum and CSF were reacted with ^{51}Cr -labeled infected and noninfected target cells in the presence and absence of guinea pig complement (lyophilized guinea pig serum from Microbiological Associates) for 1 h at 36°C. All assays were done in duplicate. At the time of assay, 80% or more of the infected target cells showed membrane stain for measles viral antigens by the direct fluorescent antibody test. Radioactivity of supernatant fluids of the cultures after incubation was determined to 3% accuracy in a Beckman 300 gamma spectrometer. Specific ^{51}Cr release was calculated as follows:

$$\% \text{ release } C_i = \left\{ \frac{\text{cpm cells}_i + S + C}{\text{total cpm}} - \frac{\text{cpm } SR_i + C}{\text{total cpm}} \right\} - \left\{ \frac{\text{cpm cells}_i + S - C}{\text{total cpm}} - \frac{\text{cpm } SR_i - C}{\text{total cpm}} \right\} \times 100$$

$$\% \text{ release } C_c = \left\{ \frac{\text{cpm cells}_c + S + C}{\text{total cpm}} - \frac{\text{cpm } SR_c + C}{\text{total cpm}} \right\} - \left\{ \frac{\text{cpm cells}_c + S - C}{\text{total cpm}} - \frac{\text{cpm } SR_c - C}{\text{total cpm}} \right\} \times 100$$

where % specific release = % release C_i - % release C_c ; cells_i, infected target cells; cells_c, control target cells; S, serum or CSF; C, complement; SR, spontaneous release; total cpm, total radioactivity of the added target cells.

Release of ^{51}Cr from target cells plus serum in the absence of C was usually less than SR, and the second set of terms in the equations then dropped out of the calculations.

Assays of HI and CF antibodies. Microtiter assays of complement-fixing (CF) and HI antibodies were performed by standard methods (14).

RESULTS

Determination of the endpoint for the CT antibody assay. Two sera, one with a low CT titer and the other with a high titer, were used

as reference sera in the various runs. Using 20% specific isotope release as the endpoint, repeated testing of the sera gave geometric mean titers (GMTs) that varied less than 1 doubling dilution between runs. The GMT $\pm$ standard deviation for the low-titer serum was $2^{5.4 \pm 0.8}$, and that for the high-titer serum was $2^{11.4 \pm 0.7}$.

With a 10% specific release as endpoint, similar repeatable results were obtained. The GMT for the low-titer serum was $2^{6 \pm 1}$, and that for the high-titer serum was $2^{12.3 \pm 1.1}$. Either 10 or 20% specific release thus provided a satisfactory endpoint for serum and CSF titrations. Five percent specific release was a less repeatable and therefore less satisfactory endpoint.

Serum antibody titer. Table 2 displays the GMTs of CT, HI, and CF antibodies for MS-definite, MS-probable, and control patients. Because the samples selected for this study were not randomly sampled from the larger study

TABLE 1. *Diagnosis of 33 control patients*

Diagnosis	No. of patients
Epilepsy	1
Lumbar disc	3
Meningitis	1
Psychological reaction	7
Amyotrophic lateral sclerosis	2
Charcot-Marie-Tooth disease	1
Radicular myeloneuropathy	1
VI nerve palsy	1
Toxic encephalopathy	1
Spastic paraparesis	1
Reactive depression	1
Guillain-Barré syndrome	1
Myelitis	1
Progressive spinal muscular atrophy	1
Right hemiparesis	1
Radiation myelopathy	1
Viral encephalitis	1
Limbic encephalitis	1
Cerebral thrombosis	1
Transient cerebral ischemia	1
Peripheral neuropathy	1
Lymphoma	1
Ependamoma	1
Adrenal myeloneuropathy	1

TABLE 2. *GMT^a of serum antibody to measles virus*

Group (no. of patients) ^b	CT (at endpoint):		HI	CF	No. of patients with CF antibody
	20% ^c	10% ^c			
MS (26)	6.1 $\pm$ 1.1 ^d	6.9 $\pm$ 1.2 ^d	8.1 $\pm$ 1.9 ^d	5.0 $\pm$ 1.1 ^e	25
Probable MS (7)	5.7 $\pm$ 0.8	6.4 $\pm$ 1.3	8.6 $\pm$ 2.0	4.9 $\pm$ 1.8	7
Controls (33)	4.8 $\pm$ 1.6	5.6 $\pm$ 1.7	6.7 $\pm$ 2.2	4.2 $\pm$ 1.4	28

^a GMT (2ⁿ) with standard deviation.

^b All had serum CT and HI antibodies.

^c Percent specific release of ^{51}Cr used for calculation of endpoint.

^d MS and probable MS > control, $P < 0.01$.

^e MS and probable MS > control, $P < 0.10$.

population, inferences from this sample to the population should only be made with caution. For this reason, we have computed measures of size of effect (η^2) (10) to help us in making statements of the importance of these sample results. We used multiple regression analysis for repeated measures (4) to examine the relationship among CF, 10% CT, and HI titers both within and between diagnostic groups. The analysis was based on a subject within groups (diagnostic category) by conditions (antibody assay) design. We found a significant gradient ($P < 0.01$) of GMTs across antibodies with CF titers lower than 10% CT titers, which were in turn lower than HI titers. This gradient was not significantly different between diagnostic groups, was quite large (accounting for over 30% of the within-subject antibody titer variance), and was very stable within the sample studied.

Comparison of diagnostic groups on different antibody assays showed that the MS patients (both MS-definite and MS-probable) had significantly higher GMTs than the control patients for CT 10%, CT 20%, and HI assays ($P < 0.01$ for each). There was also a trend for the MS groups to have higher CF titers than the control group ($P < 0.10$). These differences in antibody titer across groups were moderate in size, with the difference in CT 10% titer accounting for 16.7% and that in HI titer accounting for 12.5% of the between-subject GMT variance. These effects were also found to be stable within the sample studied.

Eighteen sera from both the MS and control groups showed a prozone at the 1:4 to 1:8 dilutions. Prozones have also been reported in CT tests with sera from patients with subacute sclerosing panencephalitis, with MS, and with natural infection (7, 11, 13).

Relationship between CSF and serum titers. The relationship between individual CT and HI titers both in the sera (Fig. 1) and the CSFs (Fig. 2) was next examined. HI serum titers were usually higher than CT titers. There was a good correlation between CT and HI titers in the serum ($r = 0.52$, $P < 0.001$) and no significant correlation evidenced in the CSF ($r = 0.17$, n.s.). The lack of a correlation in the CSF is of interest, but should be interpreted cautiously because of the possibility that at the lower dilutions the CT and HI assays may be less reliable.

Table 3 presents the serum 10% CT titers by diagnostic group in relation to the presence or absence of CSF 10% CT titers. Ten of 26 (38%) MS-definite, 4 of 7 (57%) probable-MS, and 6 of 33 (18%) control patients had detectable CT CSF antibody by the 10% endpoint. The rela-

FIG. 1. Individual serum CT antibody titers to measles virus using a 10% ^{51}Cr release as endpoint, plotted against the corresponding serum HI antibody titers. Closed circles, MS patients; closed triangles, probable MS; open circles, control patients. HI titers $>$ CT titers, $P < 0.001$.

FIG. 2. Individual CSF CT antibody titers (10% ^{51}Cr release endpoint) plotted against the corresponding CSF HI antibody titers. Closed circles, MS patients; closed triangles, probable-MS patients; open circles, control patients. No significant correlation between HI and CT titers.

tionship between serum titer and presence or absence of CSF antibody differed between MS and control groups (interaction effect) ($F = 7.2$, $P < 0.01$). Figure 3 and Table 3 display this interaction whereby, relative to combined MS and probable-MS patients, control patients without CT antibody in their CSF have lower serum CT titers than control patients with CT antibody present in their CSF. Table 3 contains similar data for HI serum antibody by diagnostic group and by presence or absence of CSF HI

antibody. No significant interaction effect was found, as can be seen by the parallel lines in Fig. 3.

Serum/CSF antibody ratios. The serum/CSF antibody ratios for CT antibodies in the MS groups were generally lower than the ratios for HI antibodies. The differences in the median and geometric mean ratios for CT antibodies were less than twofold between MS and control groups (Fig. 4).

Relation of CSF and serum CT titer to duration and severity of disease in the MS group. We found no significant correlations between either CSF or serum CT titers and either duration or severity of disease in the MS group.

TABLE 3. Relationship of height of serum GMT and occurrence of CSF antibody to measles virus

Patients (no.)	CT antibody			HI antibody		
	CSF	Serum GMT (2 ⁿ)		CSF	Serum GMT (2 ⁿ)	
		No. pos. ^a	CSF pos. ^b CSF neg. ^c		No. pos.	CSF pos. CSF neg.
MS (26)	10	7.0	6.9	13	9.4	6.8
Probable MS (7)	4	6.5	6.3	4	10.0	6.7
Controls (33)	6	7.3	5.1	7	9.1	6.0

^a Number of patients with CSF antibody.

^b Antibody present in CSF.

^c Antibody absent in CSF.

FIG. 3. Interaction effect between diagnosis, serum GMT, and presence or absence of CSF antibody to measles virus. Interaction of these factors relative to CT antibody is significantly different between control and MS groups, $P < 0.01$. There is no significant difference between control and MS groups relative to HI antibody. Solid line, MS; dotted line, probable MS; dash-and-dot line, control.

FIG. 4. Individual serum/CSF ratios of antibodies to measles virus showing the range of ratios and median ratio for each group of patients. Closed circles, MS patients; closed triangles, probable-MS patients; open circles, control patients; horizontal bars, median ratio.

DISCUSSION

There have been few studies of CT antibody in MS patients (7, 9, 12). In a report by Fuccillo et al. (9), the mean CT serum titer of MS patients was slightly less than twofold higher than mean serum titer of control patients, a significant difference at $P < 0.02$. In another study by Kratzsch and Kiessling (12), serum CT titers were similarly slightly higher in MS patients than in control patients. CT antibody in the CSF was not checked in either study. Ehrnst (7) reported CT antibody in the CSF of four MS patients despite low antibody titers to the hemagglutinin and hemolysin of measles virus.

Comparison of CT antibodies to measles virus in the CSF of MS and control patients in our study cannot be strictly made. The patients were matched with reference to time of serum and CSF sampling and with age, but could not be matched with respect to HI CSF antibody since few controls with HI CSF antibody were available. Similarly, the patients were not matched according to HI serum antibody titer since the GMT of HI antibodies was twofold lower in the control group than in the MS group. To match by serum HI antibody titer, as well as with age of donor and time of specimen sampling, was not possible with the group of available specimens. It was of interest, however, that the GMT by CT and HI was significantly lower in the control sera than in the MS group ($P < 0.01$) (Table 2). Furthermore, there was a distinctly different pattern in the serum and CSF CT antibodies as compared to HI antibodies. Thus, presence of HI antibodies in the CSF correlated with higher serum HI titers in all patients, whereas presence of CT antibodies in the CSF correlated with higher serum CT titers only in control patients (Table 3, Fig. 3). The interaction effect between diagnosis, serum CT titer, and presence or absence of CSF CT antibody was significant ($P < 0.01$). These results provide support for local central nervous system synthesis of CT antibody in MS patients, rather than passive transfer of serum antibody into the CSF because of high serum titers.

Clarke et al. (3) suggested evaluation of serum/CSF ratios of antibodies as a method to determine whether antibody synthesis is occurring within the central nervous system. By comparing serum/CSF antibody ratios to measles virus antigens and to reference antigens (adenovirus penton antigen and poliovirus) in control and MS patients, Norrby et al. (16) considered ratios of <160 to be subnormal. In our dilution series a ratio of 128 would correspond to a ratio of 160. By that criterion the 14 patients (100%) in the MS group with CT antibody in the CSF

had reduced serum/CSF ratios of CT antibodies, whereas of the 17 MS patients with HI antibody in the CSF only 7 (41%) had reduced HI antibody ratios. Five of six control patients (83%) with CSF CT antibody and three of seven (43%) control patients with CSF HI antibody had reduced ratios (Fig. 4). Three MS patients and two control patients had reduced ratios with both HI and CT antibodies. Of interest were five patients in the MS group with both antibodies in the CSF but reduced ratios only with the CT antibody. It might be expected that if antibody to more than one antigen of measles virus were synthesized within the central nervous system, the serum/CSF ratios to both should be reduced. However, such a result would depend upon the relative duration of the two antibodies in the CSF and serum. The inconsistency occurred only with CT antibody, because no patients with both antibodies in the CSF had reduced HI ratios and normal CT ratios.

The pertinent viral antigens in the measles CT test are not known, but our data suggest they may be different from the hemagglutinin. The HI test was more sensitive than the CT test in detecting serum antibody, yet there were nine CSFs with CT antibody and no detectable HI antibody (Fig. 2). Twelve CSF specimens had HI antibody but no detectable CT antibody. In a study of measles CT antibody in the sera of patients with subacute sclerosing panencephalitis, natural infection, or vaccination, it was concluded that at least two antigens of measles virus can induce CT antibody. Absorption of sera with hemagglutinin removed CT antibody in sera of vaccinees but not in sera of subacute sclerosing panencephalitis patients (11).

One hypothesis of the cause of MS suggests that an immunological attack, directed against central nervous system white matter, is stimulated by measles virus possibly latent in central nervous system tissue. In this study measles CT antibody was found more commonly in the CSFs, and in higher titer in the sera, of MS patients than in control patients. However, the appearance of measles CT CSF antibody in less than half of the patients with MS or probable MS suggests that if CT antibody is involved, it is not the sole mechanism of central nervous system destruction in MS.

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