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Antibody Titers by Mixed Agglutination to Varicella-Zoster, Herpes Simplex and Vaccinia Viruses in Patients with Multiple Sclerosis (38909)

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In the last decade, a number of sero-epidemiologic studies on multiple sclerosis (MS) have been published as summarized by Brody *et al.* (1). Significantly high titers of antibodies against measles virus have been repeatedly observed in MS sera as well as cerebrospinal fluids (CSF) (2–6). Elevated levels of antibody to other viruses such as varicella-zoster (V-Z), herpes simplex (HSV), influenza C and mumps have also been reported (3, 5, 7, 8). Recently, a high incidence of antibodies to vaccinia virus in CSF was described by Kempe *et al.* (9) but other studies failed to confirm this observation (10, 11).

In the present study, three DNA-containing viruses, V-Z, HSV and vaccinia, were selected. V-Z is responsible for a common infection of childhood, chickenpox (varicella), and HSV is one of the most prevalent viruses. Both these herpesviruses may be neurotropic and persist in a latent form for many years. Infection with vaccinia virus was also common in this country up to a few years ago because of vaccination against smallpox. Sera and CSF from MS cases were examined by mixed agglutination (MA) for antibodies to virus-specific cell surface antigen(s) produced following infection of cell cultures with these viruses. The results are compared with those obtained in normal individuals and patients suffering from other diseases. Attempts to detect antibodies to these viruses in MS brain tissue are also described.

Materials and Methods. Cell cultures. A line of human fibroblasts, FT (12), was employed for titration of antibody to V-Z surface antigen. The BIRK line of rabbit kidney cells was used for measuring antibody to HSV surface antigen (13). HEp-2 cells were selected for determination of antibody levels against vaccinia-specific surface antigen (14). FT cells were cultivated in Eagle's minimum essential medium (MEM) in Earle's saline, containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). BIRK and HEp-2 cells were grown in Eagle's basal medium in Hanks' balanced salt solution, supplemented with 10% fetal calf serum and antibiotics. After virus inoculation, all cell cultures were maintained in MEM supplemented with 3% fetal calf serum and antibiotics.

Viruses. V-Z virus, Ellen strain, HSV type 1, MacIntyre VR #3 strain, and vaccinia virus, NY strain, have been previously described (13–16).

Sera and CSF. MS sera were collected from patients in the Buffalo area in the Department of Neurology, Buffalo General Hospital, Buffalo, NY. The group consisted of 59 patients, 21 males and 38 females. Normal sera collected from healthy blood donors, kidney donors, and some laboratory personnel were supplied from collections in the Department of Microbiology. For age and sex matching studies, additional sera were obtained through Dr. R. Lambert of this department. Sera and CSF from patients with a variety of CNS diseases other than MS were provided by Dr. E. J. Manning, Dent Neurological Institute, Millard Fillmore Hospital, Buffalo, NY. Twelve CSF specimens from MS cases and 10 CSF from non-MS CNS diseases were kindly supplied by Dr. R. M. Herndon, Department of Neurology, The Johns Hopkins University, Baltimore, MD. Sera from kidney diseases, rheumatoid arthritis and pregnant women were from collections in the Department of Microbiology. Sera and CSF from patients

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suffering from suspected virus diseases were provided by the Erie County Virology Laboratory, Buffalo, NY. Sera of sarcoidosis and asthma patients were received from Dr. R. E. Reisman, Allergy Research Laboratory, Buffalo General Hospital.

MA test. MA test was performed essentially by the procedure for the detection of surface antigens produced on virus-infected cells (13–15). In the present experiments the indicator cell suspension consisted of sheep erythrocytes (SRBC) sensitized by monkey anti-SRBC serum and agglutinated by goat anti-human globulin serum (Grand Island Biological Co., Grand Island, NY).

FT, BIRK and HEp-2 cells grown in Kahn tubes (12 × 75 mm) were infected with V-Z, HSV or vaccinia viruses respectively. The infected cell monolayers were exposed to sera or CSF at various dilutions for 90 min at 25°. Sera and CSF were usually diluted at semi-$\log_{10}$ intervals and in some tests conventional twofold dilutions were used. After washing, the indicator cell suspension was added to each tube. Adherence of indicator cells was observed under the low power of the microscope and positive reactions were graded from 1+ to 4+, depending upon the degree of adherence. The reciprocal of the highest dilution yielding at least a 2+ reaction was selected as the end point titer.

Washing fluid of brain tissue. Brain slices obtained from autopsies of MS patients were supplied by Dr. W. W. Tourtellotte, VA Wadsworth Hospital Center, Los Angeles, CA. A normal brain was provided by the Veterans Administration Hospital, Buffalo, NY. Slices of MS brains were dissected to dissociate plaque areas from nonplaque areas, pooled separately, and coded as "MS lesion" and "MS non-lesion". Brain tissue was minced and homogenized in saline using an Omnimixer to form a 10% (v/v) suspension. This suspension was centrifuged at 5000 rpm for 30 min in a refrigerated Sorvall RC2-B centrifuge and the tissue sediment was washed several times in saline until a clear supernatant was obtained. Supernatant decanted after the first centrifugation of the suspension and those collected after each washing were pooled and concentrated about 10 times by dialysis against sucrose. The preparation was then dialyzed for two days against phosphate buffered saline, pH 7.2.

Results. Antibody titers to V-Z, HSV and vaccinia viruses as determined by MA are shown in Table I. The geometric mean titer (GMT) of antibodies for V-Z in the 59 MS patients was significantly higher than the GMT in normal individuals as well as in non-MS patients with the exception of rheumatoid arthritis. Of interest is the fact that in only one case of MS was a history of zoster elicited. Antibodies for vaccinia virus had also a significantly higher GMT in MS patients than in other subjects with the exception of individuals suspected of viral diseases. The GMT of HSV antibodies was not higher in MS patients than in other groups.

A comparison of antibody titers in sera from MS cases and normal individuals residing in the Buffalo area, matched for sex and age, is shown in Fig. 1. The GMT for V-Z in MS was 3.69 ± 0.05 as compared to 2.76 ± 0.07 in normal individuals ($p < 0.001$). For vaccinia, the GMT in MS was 3.41 ± 0.07 and in normal subjects, 3.01 ± 0.08 ($p < 0.001$). In the case of HSV, the GMT in MS was 3.17 ± 0.15 and in normal persons it was 3.14 ± 0.15 ($p > 0.8$).

We also had the opportunity to study CSF from patients with MS and non-MS CNS diseases collected in Baltimore and Buffalo (Table II). CSF from patients suspected of viral CNS diseases collected in Buffalo were also available. In the Baltimore material, antibody titers against the three viruses tested were higher in MS than in the non-MS group. The reason for the low incidence of antibodies to HSV in the 10 CSF collected from non-MS cases of CNS diseases in Baltimore is not clear.

Washing fluids obtained from a pool of brain tissue collected from MS cases were tested for antibodies. Similar preparations from a normal brain were also studied as controls. As shown in Table III, positive reactions were obtained in the MA test with washing fluid obtained from lesion as well as non-lesion areas. Washing fluid from normal brain had some antibody activity

TABLE I. ANTIBODY TITERS TO VARICELLA-ZOSTER, HERPES SIMPLEX AND VACCINIA VIRUSES DETERMINED BY MIXED AGGLUTINATION.

Sera	Number of individuals	Varicella-Zoster			
		% Pos.[a]	GMT[b]	SE[c]	P[d]
1. Multiple sclerosis	59	100.0	3.68	±0.04	
2. Non-MS CNS diseases	89	94.2	2.91	±0.06	<0.001
3. Various kidney diseases	17	88.0	2.50	±0.16	<0.001
4. Rheumatoid arthritis	24	100.0	3.48	±0.09	<0.05
5. Suspected viral CNS disease	20	84.0	3.08	±0.21	<0.001
6. Asthma	11	91.0	2.59	±0.16	<0.001
7. Sarcoidosis	11	91.0	2.90	±0.12	<0.001
8. Pregnancy	31	80.5	2.65	±0.13	<0.001
9. Normal	64	98.4	2.77	±0.06	<0.001

	Herpes simplex				Vaccinia			
	% Pos.	GMT	SE	P	% Pos.	GMT	SE	P
1.	74.6	3.20	±0.14		98.3	3.41	±0.07	
2.	86.5	3.41	±0.10	>0.1	84.1	2.79	±0.08	<0.001
3.	70.5	3.24	±0.29	>0.2	82.0	2.41	±0.17	<0.001
4.	100.0	3.73	±0.07	<0.05	87.0	2.90	±0.14	<0.001
5.	63.1	3.08	±0.23	>0.1	84.0	3.05	±0.20	<0.05
6.	ND[e]	—	—	—	91.0	2.59	±0.15	<0.001
7.	ND	—	—	—	82.0	2.68	±0.18	<0.001
8.	58.0	2.73	±0.20	<0.05	83.8	2.66	±0.13	<0.001
9.	78.0	2.95	±0.12	>0.1	93.8	2.98	±0.08	<0.001

[a] Mixed agglutination titer $\log_{10} \geq 2.0$.

[b] Geometric mean. Sera yielding negative reactions at the lowest dilution tested, 10^{-2} were assigned titer of 1.5.

[c] Standard error.

[d] Significance of GMT differences between multiple sclerosis and other categories. Probability was calculated by *t* test.

[e] Not done.

for HSV but negative results were obtained with V-Z and vaccinia.

Discussion. A higher incidence of V-Z antibody in sera of MS patients was described by Ross *et al.* (7). They found that V-Z antibody was detected in 84% of MS whereas only 60% of control sera were positive. Ross and his associates (8) also reported that V-Z antibody titers of 13 MS sera were significantly higher than those of sera from 17 cases of retrobulbar neuritis. A constantly higher level of antibody to V-Z in MS sera than in controls was also found by Brody *et al.* (3). In recent studies, Haire *et al.* (5, 17) found no differences in V-Z antibody levels between MS and controls whereas antibody titers to both measles and HSV were significantly elevated in MS sera. In all of the studies cited, V-Z antibody was measured by the complement fixation test except in the last in which immunofluorescence was employed.

Other data have also been provided which suggest the role of V-Z in MS. Lenman (18) noted that significantly more MS patients had a past history of herpes zoster than the control group studied. In Lenman's group, 10 of 50 MS cases reported a history of zoster whereas one of 59 cases in our group was associated with zoster.

In our studies reported here, the GMT for V-Z antibody in MS was 8.5 times higher than in matched control subjects. We used the mixed agglutination test which is very

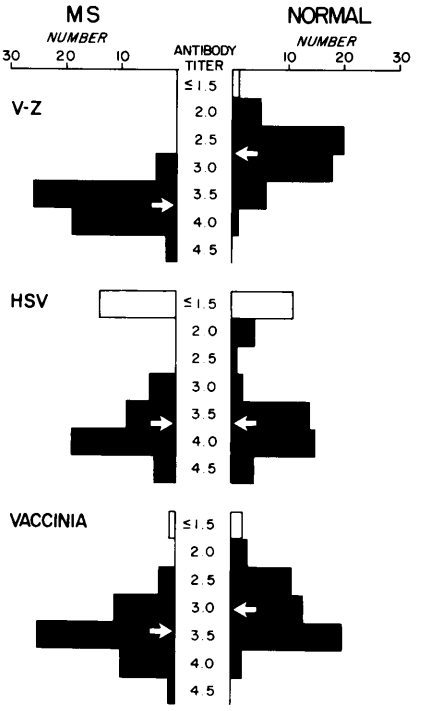

FIG. 1. Antibody titers to varicella-zoster (V-Z), herpes simplex (HSV) and vaccinia viruses were determined in sera from patients with multiple sclerosis (MS) and normal individuals, Buffalo, NY, matched for sex and age. The number of individuals studied in each group was 51. Antibody titers ($\log_{10}$) were determined by MA test. Open block represents number of sero-negative individuals (titer <2.0) and solid block represents number of sero-positive individuals (titer ≥2.0). Geometric mean antibody titer for each virus is shown by arrow.

sensitive for the detection of antibodies to viral antigens appearing on the cell surface. It has been documented for HSV (13) and vaccinia (14) that these antigens belong to the "early" antigens which appear on the surface of infected cells prior to viral DNA synthesis. It is likely that similar antigens are synthesized in cells infected by V-Z. Antibodies to these cell-surface viral antigens might be found in individuals in whom defective virus replication has occurred.

While our results draw attention to V-Z, consideration must also be given to vaccinia since antibody levels to this virus were also elevated in MS sera and CSF in comparison to controls. In this regard our results would support the suggestion of Kempe *et al.* (9) that vaccinia virus may be involved in the etiology of MS.

Among the other groups tested, rheumatoid arthritis deserves comment in view of the high antibody titers to V-Z and HSV. On the other hand, in this group vaccinia antibody titers were in the normal range. Results obtained with rheumatoid arthritis sera collected in Buffalo could be considered as a form of "internal control" since the pattern of results was different for this chronic disease than for MS. The significance of this finding requires more data before valid interpretation may be made.

Although the material studied was quite limited, antibodies to V-Z, HSV and vaccinia were detected in washing fluids from

TABLE II. ANTIBODY TITERS IN CEREBROSPINAL FLUIDS TO VARICELLA-ZOSTER, HERPES SIMPLEX AND VACCINIA VIRUSES DETERMINED BY MIXED AGGLUTINATION (MA).

		Varicella-Zoster		Herpes simplex		Vaccinia	
	No. patients	%Pos.[a]	MA[b] titer ($\log_{10}$)	%Pos.[a]	MA[b] titer ($\log_{10}$)	%Pos.[a]	MA[b] titer ($\log_{10}$)
Multiple sclerosis							
Baltimore	12	75.0	1.29	75.0	1.38	75.0	1.04
Buffalo	3	67.0	0.83	33.0	0.65	0	0.50
Non-MS CNS disease							
Baltimore	10	10.0	0.60	10.0	0.65	40.0	0.90
Buffalo	88	23.9	0.69	68.0	1.10	27.5	0.65
Suspected viral CNS disease							
Buffalo	45	48.9	1.00	57.8	1.11	53.3	0.88

[a] Titer $\log_{10} \geq 1.0$.

[b] Geometric mean. CSF yielding negative reactions at the lowest dilution tested, 10^{-1} were assigned titer of 0.5.

TABLE III. ANTIBODIES IN WASHING FLUIDS OF BRAIN TISSUE TO VARICELLA-ZOSTER, HERPES SIMPLEX AND VACCINIA VIRUSES DETERMINED BY MIXED AGGLUTINATION (MA).

Washing fluids	Protein concentration (mg/ml)	MA titer[a] V-Z	HSV	Vaccinia
Multiple sclerosis				
Lesion	6.3	80	20	80
Non-lesion	4.6	40	20	20
Normal brain	3.3	<1	8	<1

[a] Highest dilutions yielding reaction ≥2+.

brain tissues of MS patients. A washing fluid prepared from a normal brain showed only low titer of antibody to HSV.

Sero-epidemiologic studies have implicated a number of viruses in the etiology of MS. One could conclude from these numerous reports that such studies have limited value in unraveling the etiology of MS. The high levels of antibodies may only be indicative of a "disturbed" immunological apparatus and thus elevated antibody titers may be found for a number of viruses unrelated to the disease. However, another point of view also emerges. It might well be that, depending on genetic factors, geographic region, age, etc., MS may have a multiple viral etiology.

Summary. Antibody titers to varicella-zoster (V-Z), herpes simplex (HSV) and vaccinia viruses were determined in sera collected in Buffalo, NY, from patients with multiple sclerosis (MS), patients with other diseases, and normal individuals. The mixed agglutination test employing cell cultures infected with one of the above viruses as a source of antigen was used to determine antibody titers. The geometric mean titer (GMT) for V-Z in 59 MS cases was significantly higher than that observed in patients with other diseases and in normal individuals. The GMT for vaccinia was also higher in MS patients but the difference was not as great as for V-Z. No difference was observed in the titer of antibodies for HSV. Similar results were obtained for 51 MS patients compared to healthy controls matched for sex and age. Higher antibody titers for V-Z and HSV were observed in cerebrospinal fluids from MS cases than in those from non-MS CNS patients in Baltimore, MD. Antibodies to V-Z, HSV and vaccinia were detected in washing fluids from brain homogenates of MS cases.

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