Sensitivity of Different Assay Systems for Immunoglobulin M Responses to Varicella-Zoster Virus in Reactivated Infections (Zoster)

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An immunoglobulin M response to varicella-zoster virus was detected in 70% of zoster patients by solid-phase radioimmunoassay, in 52% by indirect immunofluorescence, in 48% by neutralization on sucrose density gradient fractions, and in 27% by an antibody class capture enzyme immunoassay. The patients showed marked variations in their varicella-zoster virus immunoglobulin M responses detectable in the different assays.

The frequency with which an immunoglobulin M (IgM) response to varicella-zoster virus (VZV) has been demonstrated in reactivated infections (zoster) has varied widely in different studies with a variety of methods for antibody detection (1-5, 8-10). Virus-specific IgM antibody was detected in slightly over 80% of zoster patients in studies using an antibody class capture radioimmunoassay (RIA) procedure (10) or an antibody class capture enzyme immunoassay method with an enzyme-labeled antigen (9). A solid-phase RIA demonstrated VZV IgM antibody in 69% of patients with zoster (1), and immunofluorescence (IF) staining procedures (4, 5) and a four-phase antibody class capture enzyme immunoassay (ACCA) (3) demonstrated VZV IgM antibody in approximately 50% of zoster patients studied. Neutralizing antibody assays with IgM antibodies separated by sucrose density gradient centrifugation detected VZV IgM in only 1 of 22 zoster patients (8).

Direct comparative studies of different methods for the detection of VZV IgM antibody in zoster patients have not been reported, and some of the previous studies did not take into account the fact that rheumatoid factor may give false-positive results for viral IgM antibody. In an effort to obtain more definitive information about the frequency of an IgM response in reactivated VZV infection and to better evaluate the relative sensitivity of different assay methods for the detection of VZV IgM in zoster, direct comparative studies were done by using four methods. These included neutralization with IgM antibody fractions separated by sucrose density gradient centrifugation (8), a solid-phase RIA (1), indirect IF staining (6, 7), and an ACCA (3). The specificity of these assays for VZV IgM was demonstrated in previous studies in which they gave negative results for patients with other viral infections, including herpes simplex virus infections (1, 3, 7, 8).

Sera from 33 patients with zoster were examined. All of the patients were 18 years of age or older. For 23 patients, VZV infection was confirmed by detection of VZV antigen in vesicular lesion smears; fluorescein-conjugated antibodies from a VZV immune monkey serum were used (6), and a conjugate to herpes simplex virus served as a control. VZV infection in the other 10 patients was confirmed by a diag-

nostically significant antibody titer rise by complement fixation or indirect IF staining or both. Twenty-three of the patients had lymphoma but uncomplicated zoster, and they showed immune responses paralleling those of normal zoster patients. Paired sera were available for 27 patients; acute-phase specimens were collected between 2 and 8 days postonset, and convalescent-phase specimens were collected between 6 and 30 days postonset. Single convalescent-phase serum samples collected between 14 and 30 days postonset were examined for six patients.

Neutralization tests on serum fractions separated by sucrose density gradient centrifugation were performed as previously described (8), with approximately 10 hemolytic units of guinea pig complement in the serum-virus mixtures. All IgM fractions were shown by immunodiffusion to be free of contaminating IgG or IgA. The solid-phase RIA was performed as previously described (1), and sera giving positive results were checked for the presence of rheumatoid factor. For indirect IF (6) and the ACCA (3), sera were adsorbed with aggregated human IgG to remove rheumatoid factor.

The abilities of the four test procedures to detect VZV IgM in one or both serum specimens from the zoster patients are shown in Table 1. The RIA detected VZV IgM in approximately the same percentage of zoster patients as in a previous study (1). The percentage of patients showing VZV IgM by indirect IF staining was comparable to that seen in studies reported by other workers (4, 5). The percentage of zoster patients showing VZV IgM in neutralization tests was markedly higher than that reported in an earlier study (8). The fact that serum fractions were examined at an initial dilution of 1:2 in the present study and 1:4 in the earlier study may account for the higher proportion of positive results. The ACCA detected the fewest VZV IgM responses in the zoster patients. Positive antibody titers were very high by RIA, very low by neutralization, and intermediate by IF and ACCA (Table 1).

It is noteworthy that relatively few patients showed positive results for VZV IgM in all of the test systems or even in three of the four systems (Table 2). The combinations of tests giving positive results varied widely among the patients (Table 2). Of 12 patients (data not shown) who were tested in only three assays, 6 were positive in one or more tests, but

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TABLE 1. Comparison of four assays for detection of VZV IgM in zoster patients

Test	No. of patients positive/no. tested	% Positive	Range of positive titers	Mean positive titer
RIA	23/33	70	256 ^a –2.6 × 10 ⁵	70,550
IF	17/33	52	8–2,048	117
Neutralization	10/21	48	2–16	6
ACCA	9/33	27	8–4,096	595

^a Reciprocal of titer.

only 1 was positive in all three, 1 was positive in two (IF and RIA), and 4 were positive in a single test (3 by RIA and 1 by IF). Two patients were negative for VZV IgM in all four test systems, and eight were negative in all three of the systems in which they were tested (RIA, IF, and ACCA). Correlation between results in two test systems was 71% between neutralization and ACCA, 70% between RIA and IF and between IF and ACCA, 62% between neutralization and IF, 58% between RIA and ACCA, and 52% between RIA and neutralization.

The ability of the four tests to detect VZV IgM at different times after the onset of zoster is shown in Table 3. The RIA gave more positive results than did the other tests with early sera collected 2 to 5 days postonset. However, with sera collected at 6 to 10 days after onset, the RIA and IF gave comparable numbers of positive results. The RIA gave twice as many positive results with sera collected at 11 days or later than with sera collected at 6 to 10 days postonset. With one exception, the neutralization test was positive only with sera collected between 11 and 30 days after onset.

These comparative studies confirmed that an IgM response to VZV occurs in a fairly high proportion of zoster patients and showed that different assay systems vary markedly in their ability to detect the response. The studies also showed that positive results in the different test systems vary widely among zoster patients. The RIA detected VZV IgM in the greatest number of zoster patients, and for six patients it was the only positive test. Even with the most sensitive test system, IgM responses were not detected in a high enough proportion of patients to make the IgM assay a particularly helpful approach to zoster serodiagnosis. However, one situation in which IgM assays might be useful is in differentiating between zoster and herpes simplex virus infections if it is not possible to isolate virus or to demonstrate antigen in lesion specimens; IgM antibody responses

TABLE 2. Combinations of positive tests for VZV IgM in 19 zoster patients^a

Positive results in:	Positive tests (no. of patients)
All four tests	ACCA, IF, neutralization, RIA (5)
Three tests	ACCA, IF, RIA (2) IF, RIA, neutralization (2) ACCA, RIA, neutralization (1)
Two tests	IF, RIA (4) IF, neutralization (1) RIA, neutralization (1)
One test	RIA (3)

^a Nineteen of 21 patients tested in all four assays and positive in one or more.

TABLE 3. Comparison of four assay systems for detection of VZV IgM in zoster patients by time after onset

Days postonset	No. of serum samples tested	No. (%) of samples positive by:			
		RIA	IF	Neutralization	ACCA
2–5	27	7 (26)	1	0	1
6–10	16	7 (44)	8 (50)	1 (20)	3
11–30	17	15 (88)	11 (65)	9 (53)	7 (41)

to VZV do not show the heterotypic reactivity between VZV and herpes simplex virus that is sometimes seen in IgG antibody responses (7–9).

Although the immunoassays used in this study were able to detect VZV IgM in some sera that were negative in neutralization tests, this does not appear to be entirely a reflection of greater sensitivity of the immunoassays, because immunoassays were negative for some sera that were positive by neutralization. Of the 10 sera positive for VZV IgM by neutralization, 1 was negative by RIA, 2 were negative by IF, and 4 were negative by ACCA. The immunoassays used in this study employed crude antigens or infected cells and thus would be expected to detect antibodies to a variety of viral antigens. Neutralization, however, would detect only those antibodies which react with or block sites on the virus which are critical in the initiation of infection. This may account for some of the discrepant results seen between neutralization and immunoassays.

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