JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1982, p. 878–884
0095-1137/82/110878-07$02.00/0

Vol. 16, No. 5

Comparison of the Raji Cell Line Fluorescent Antibody to Membrane Antigen Test and the Enzyme-Linked Immunosorbent Assay for Determination of Immunity to Varicella-Zoster Virus

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Received 1 April 1982/Accepted 25 June 1982

A prospective study was performed comparing the fluorescent antibody to membrane antigen (FAMA) test and the enzyme-linked immunosorbent assay (ELISA) for identifying susceptibility and seroconversion to varicella-zoster virus (VZV) infection. A total of 75 sera were collected from index cases and from sibling and parent contacts in 10 families. Varicella-zoster virus-infected human diploid embryonic fibroblasts and continuous lymphoblastoid cells (Raji cells) were compared as indicator cells in the FAMA test. Equivalent results were obtained with both types of cell. Results of the FAMA test and the ELISA were identical in two ways. (i) The same 11 individuals were initally defined as susceptible (seronegative), and 9 of them (82%) developed fourfold rises in antibody titers, clinical varicella, or both. (ii) Of 21 immune (seropositive) individuals, 4 developed fourfold antibody rises by FAMA tests, and 3 of these 4 responded by ELISA. Infection was asymptomatic in these individuals. The geometric mean titer by ELISA was significantly higher than by the FAMA test. The results indicated that the ELISA and the FAMA test have similar capacities to define susceptibility to varicella-zoster virus and that subclinical infection with varicella-zoster virus may be common.

The fluorescent antibody to membrane antigen (FAMA) assay for detecting immunoglobulin G (IgG) antibody to varicella-zoster virus (VZV) was first described by Williams et al. (11) in 1974. It soon became apparent that the FAMA was a notable advance in the serodiagnosis of VZV infections since it reliably differentiated susceptible and immune individuals and was specific even at very low serum dilutions (5, 10, 12). The test has since become a preferred method for definitive serodiagnosis of VZV infection. More recently, the enzyme-linked immunosorbent assay (ELISA) has also been shown to be equal to or better than such standard assays as complement fixation, neutralization, and immune adherence hemagglutination for detecting IgG antibodies to VZV (3). The FAMA test and the ELISA have been directly compared in only one previous study, by Shanley et al. (8). In that study, the FAMA test and the ELISA were comparable in specificity but not in sensitivity. For reasons related to cost, ease and rapidity of performance, and broader utilization, it is desirable for equivalent tests for serodiagnosis of VZV infection to be defined.

In comparison with most cell types, human diploid embryo fibroblasts (HDEF) are highly susceptible to VZV infection and are the cell type most often used in FAMA tests. Levonton-Kriss and co-workers (7) have shown that VZV can infect the continuous lymphoblastoid cell line, Raji. Moreover, they demonstrated VZV antigen in the membranes of infected Raji cells by indirect immunofluorescence. In this study, we evaluated the FAMA test with VZV-infected Raji cells and the ELISA in comparison with the standard FAMA test with fibroblast cells for defining susceptibility to infection with VZV. Our results indicated that VZV-infected Raji cells were an equivalent substitute for HDEF cells in the FAMA test. Also we report that our ELISA was as sensitive and specific as the FAMA test for the determination of immune status.

MATERIALS AND METHODS

Patients and sera. Pediatric patients in the Kaiser-Permanente health care program were identified as

† Deceased.

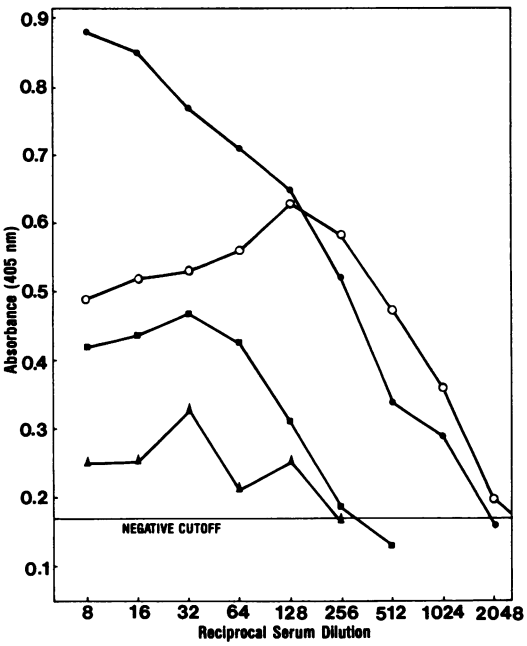

FIG. 1. The effect of serum dilution on VZV IgG antibody reactivity to VZV antigen in ELISA. The representative titration curves of four different serum samples are illustrated. The solid horizontal line represents the absorbance value differentiating positive and negative results as defined in the text.

having varicella based on the appearance of typical rash and fever without any history of varicella. Serum specimens were collected at that time (within 3 days of onset), and if parental consent was given, they were also collected from index cases, parents, and siblings. Approximately 3 weeks later, a second serum specimen was drawn from each subject. If varicella developed in a sibling contact, a second and sometimes a third serum specimen was obtained in each case 3 to 7 weeks later. Sera were stored at −20°C until the time of testing. Sera collected from siblings ≥7 days before the onset of clinical varicella (and at time of onset in index cases) were used as negative controls in each assay. Varicella zoster immune globulin (Massachusetts Public Health Biologics Laboratories, Boston, Mass.) was used as a positive control. Comparability of results of individual FAMA assays was controlled by demonstrating that this immune globulin yielded the same titer in each assay.

VZV infection of Raji cells and fibroblasts. HDEF (Flow 5000, Flow Laboratories, McLean, Va.), MRC-5 cells (MA Bioproducts, Walkersville, Md.), and Raji cells were grown in Eagle minimal essential medium (HDEF, MRC-5) and RPMI 1640 (Raji) with 10% fetal bovine serum. VZV isolates, Stevens and CaQu, were propagated by the passage of cell-associated virus as previously described (6). Raji cells were infected by cocultivation of 5×10^7 cells per 150-cm^2 flask of VZV-infected HDEF displaying 90% cytopathic effects. After 2 h at 37°C, unattached Raji cells and media were removed, and fresh medium was added. The VZV-infected Raji cells remained in contact with the infected HDEF for 24 h and were dispersed into the medium by standing the flasks upright and tapping them. The medium and Raji cells were then removed. Infected HDEF were not removed by this procedure, and virtually 100% of the Raji cells infected in this manner contained VZV membrane antigen as detected by the FAMA test. The VZV-infected Raji cells were frozen in liquid nitrogen in medium with 20% fetal bovine serum and 10% dimethyl sulfoxide for later use.

FAMA test. The FAMA assay was carried out by the procedure of Zaia and Oxman (13) with glutaraldehyde-fixed VZV-infected HDEF or unfixed VZV-infected Raji cells. Serum dilutions were mixed in plastic 96-well tissue culture trays (MA Bioproducts) with 5×10^4 cells, 25 μl of each, and incubated for 30 min at room temperature. Cells were then washed twice in 0.1 ml of 0.1% gelatin-saline. Microtiter plates were then centrifuged at 400 × *g* for 5 min, and the wash supernatant fluid was carefully removed. Cells were suspended in 25 μl of fluorescein-conjugated goat anti-human IgG (Cappel Laboratories, Inc., West Chester, Pa.) for 30 min at room temperature and were washed three times as before. Cells were suspended in 25 μl of 50% glycerol-saline and were observed in dark-field illumination with a UV high-pressure mercury lamp. A complete rim of fluorescence on no fewer than 30% of the cells was scored as a positive test.

ELISA. The microtiter plate procedure of Voller et al. (9) was used with modifications. Polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated overnight at 4°C with VZV antigen diluted in bicarbonate-carbonate buffer (pH 9.6). For antigen preparation, VZV-infected HDEF cells were harvested by scraping when they displayed 4+ cytopathic effects. Cells were washed three times in 0.1 M Tris-buffered saline (MA Bioproducts), and after the final wash the pellet was suspended to 0.5 ml of Tris-buffered saline per 150-cm^2 flask and sonicated. The lysate was centrifuged at 3,000 rpm for 15 min, and the supernatant was used as VZV antigen. Uninfected HDEF antigen was prepared in a like manner, and the protein concentration was determined and used in a concentration equivalent to that of VZV antigen. The ELISA procedure with goat anti-human IgG alkaline phosphatase conjugate (MA Bioproducts) was carried out as previously described (2). Serum dilutions were made in microtiter plate wells coated with either VZV antigen or HDEF antigen. Background absorbance occurring in the control well was subtracted from the reading obtained in the VZV antigen-coated wells. Absorbance was measured at 405 nm with a TiterTek Multiskan (Flow Laboratories). The cutoff value for establishing serum antibody positivity and titration endpoints was determined by assaying in duplicate 15 preinfection sera from preinfection and convalescent VZV serum pairs. The mean value plus two standard deviations obtained at serum dilutions of 1:8 was 0.165. Not all sera were assayed on the same day, so to monitor day-to-day reproducibility, the same three sera (one negative, one low positive, and one high positive) were diluted to endpoint and included in each ELISA. Variations in serum endpoint titers greater than twofold were not observed for any of the three sera in 10 different tests.

TABLE 1. Comparison of ELISA and FAMA test for detecting serum IgG antibody to VZV for serodiagnosis and immune status determination

Family	Individual[a]	Serum sample[b]	Varicella infection		Day of bleed[c]	IgG titer	
			Current	Past		FAMA	ELISA
I	Index	A1	+	−	+1	<2	8
		A2			+21	128	256
	Sibling	B1	−	−		<2	<8
		B2				<2	<8
		B3				<2	<8
	Sibling	C1	+	−	−14	<2	<8
		C2			+6	256	2,048
	Mother	D1	−	+		8	256
		D2				8	256
		D3				16	256
	Father	E1	−	?		32	1,024
		E2				32	1,024
		E3				32	1,024
II[d]	Sibling	B1	+	−	−12	<2	<8
	Mother	C1	−	+		16	512
		C2				16	1,024
III	Index	A1	+	−	+2	8	<8
	Father	B1				8	32
		B2				64	256
	Mother	C1	−	+		16	128
		C2				64	1,024
IV	Index	A1	+	−	+3	<2	<8
		A2			+38	128	2,048
	Sibling	B1	−	−		64	2,048
		B2				64	1,024
	Mother	C1	−	+		16	1,024
		C2				16	512
	Father	D1	−	+		16	2,048
V[d]	Sibling	B1	−	−		16	512
		B2				8	512
	Sibling	C1	+	−	−8	<2	<8
		C2			+44	128	2,048
VI[d]	Sibling	B1	−	+		128	8,192
		B2				256	8,192
	Sibling	C1	−	+		16	2,048
		C2				32	2,048
	Sibling	D1	−	+		8	1,024
		D2				16	2,048
	Sibling	E1	−	+		16	2,048
		E2				16	1,024

TABLE 1—*Continued*

Family	Individual[a]	Serum sample[b]	Varicella infection		Day of bleed[c]	IgG titer	
			Current	Past		FAMA	ELISA
	Mother	F1	−	+		16	1,024
		F2				16	2,048
	Father	G1	−	?		16	2,048
		G2				16	2,048
VII	Index	A2	+	−	+39	256	4,096
	Sibling	B1	+	−	−13	<2	<8
		B2			+24	128	1,024
	Sibling	C1	−	−		16	512
		C2				8	512
	Mother	D2	−	+		8	128
	Father	E2	−	+		8	512
VIII	Index	A2	+	−	+30	128	1,024
	Sibling	B1	+	−	−14	<2	<8
		B2			+16	512	512
	Sibling	C1	+	−	−14	<2	<8
		C2			+16	256	2,048
	Sibling	D1	+	−	−16	<2	<8
		D2			+14	256	1,024
	Mother	F1	−	+		16	128
		F2				128	1,024
IX	Index	A2	+	−	+18	128	2,048
	Mother	B1	−	+		16	4,096
		B2				8	2,048
	Sibling	C1	+	−	−9	<2	<8
		C2			+8	≥256	1,024
	Father	D1	−	+		8	1,024
X	Index	A1	+	−	+2	<2	<8
	Sibling	B1	−	−		<2	<8
		B2				128	1,024
	Sibling	C1	−	−		<2	<8
		C2				<2	<8
	Mother	D1	−	+		16	256
		D2				64	512
	Father	E1	−	?		4	128
		E2				4	128

[a] Index means the individual in the family who first contracted varicella.
[b] Numbers 1, 2, and 3 indicate first, second, or third bleed.
[c] Number of days before (−) or after (+) varicella rash in those individuals who contracted varicella.
[d] No bleed made in index case.

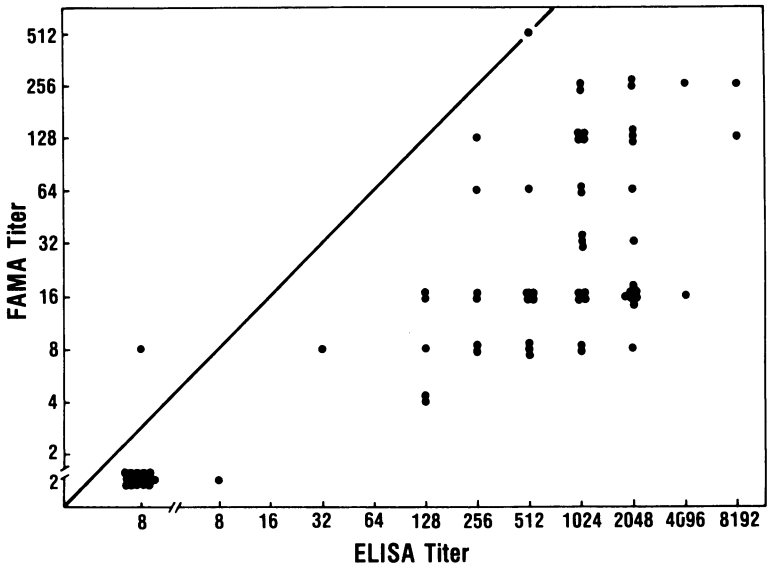

FIG. 2. Comparison of serum VZV antibody titer as determined by ELISA and FAMA tests. Titers <8 and <2 were considered negative in the ELISA and the FAMA test, respectively.

RESULTS

Routine use of the FAMA test for determining antibody to VZV involves the maintenance of diploid fibroblasts and VZV in the laboratory. Lymphoblastoid Raji cells can be handled more easily in the laboratory since they are a continuous cell line and cell dispersal by enzymatic or mechanical methods is not necessary. In addition, we observed that the problem of cell clumping or periphery distortion found to occur with unfixed VZV-infected HDEF (13) did not occur with previously frozen unfixed Raji cells. Because of differences in cell size, FAMA tests with HDEF were routinely read at a magnification of ×200, and tests with Raji cells were read at ×400. Another advantage of using Raji cells was that the number of Raji cells that could be infected with each flask of infected fibroblasts was three to five times the number of fibroblasts. Thus, the number of diploid cell cultures used was substantially reduced.

We tested 45 of the 75 sera available from this study with Raji cells and HDEF, and we found similar geometric mean serum titers in both (12.73 ± 6.73 and 14.83 ± 7.11, respectively). Moreover, we found a complete concordance of sera defined as positive or negative for the presence of antibodies by the two tests. The remaining sera were, therefore, tested with Raji cells only, and the results obtained with Raji cells are presented here.

Representative ELISA titration curves showing the reaction of antigen with increasing serum dilutions are shown in Fig. 1. A prozone-like effect was frequently but not invariably observed with sera titrated by ELISA. This was evident from the fact that absorbance increased with greater serum dilution before it decreased steadily with further serum dilution. There was no correlation of this observation with serum titer or with whether the infection had been recent or remote. The reason for this prozone-like phenomenon is not clear. Table 1 summarizes the results of FAMA and ELISA tests to detect IgG antibody to VZV in the 75 sera from members of the 10 families. The geometric mean titer obtained by ELISA (mean ± standard deviation, 235.57 ± 11.88) was significantly higher than that obtained by FAMA testing (14.22 ± 6.23). However, the sensitivity in terms of the percentage of sera positive for antibodies was similar, although the ELISA consistently gave higher endpoint titers (Fig. 2). The results differed with only two serum samples, IA1 and IIIA1. Since these sera were obtained 1 or 2 days after the appearance of vesicular rash, the results were consistent with the detection of low levels of developing antibodies by one or the other test but not by both. These times of appearance of VZV-specific IgG were earlier than those reported in previous studies, where IgG was not detected until 5 days after the onset of rash (1, 4).

One or more serum specimens from 8 of 10 index cases were tested, and antibodies were detected by both the FAMA test and the ELISA in at least one specimen in all cases. Paired acute- and convalescent-phase sera were obtained in two index cases (IA and IVA), and

both showed antibody rises greater than fourfold. A total of 18 siblings were included in the study group, 11 of whom were seronegative (susceptible) at the time of the onset of symptoms in the index case. Of 11 seronegative subjects, 9 (82%) developed clinical varicella, but none of the 7 seropositive siblings developed clinical or serological evidence of infection. All 16 parents tested had antibodies in the first serum sample tested, and none developed clinical varicella. Paired sera were available from 12 of these parents and 7 previously immune siblings. Three developed antibody titer rises of fourfold or more by both the FAMA test and the ELISA, and one additional parent had a fourfold rise by the FAMA test and a twofold rise by the ELISA. FAMA tests were performed on these specific sera with both Raji and HDEF cells, and identical results were obtained with each. Thus, fourfold antibody rises indicating probable subclinical reinfection were seen in 4 of 19 (21%) family members (parents and siblings) with antibodies before exposure.

DISCUSSION

The FAMA test has previously been established as a sensitive and specific assay for detecting IgG antibodies to VZV (11, 13). The test is particularly useful because the absence of antibodies by the FAMA test indicates susceptibility to VZV, and conversely, the presence of antibodies indicates immunity. Second, seroconversion demonstrated by the FAMA test is a sensitive and specific indicator in ordinary circumstances of the occurrence of infection. Unfortunately, the FAMA test is not entirely suitable for routine diagnostic virology since tissue culture capabilities are required and microscopic reading of each specimen is time-consuming and subjective. Moreover, the use of diploid cells in the assay is relatively expensive, and the slow growth and limited life span of these cells add to the difficulties involved in performing the assay. In this study, two alternative approaches were evaluated, namely the ELISA and the FAMA test with Raji cells used as indicator cells. The ELISA can be performed rapidly and easily, and it uses objective endpoint determinations. Moreover, ELISA VZV antigen can be easily prepared from cell lysates. The Raji cell line used in the alternative FAMA test is widely available and is a rapidly growing continuous line.

The experimental design in this study involved the use of sera from index cases and from sibling and parent contacts. With this approach, it was possible to confirm the susceptibility of certain individuals based on subsequent development of clinical varicella. By this method, the FAMA test with Raji cells and the ELISA were both defined as sensitive and specific. Seronegativity correlated with susceptibility since 90% of the susceptible siblings developed clinical varicella or seroconversion, and seropositivity correlated with resistance to clinical varicella. An excellent concordance of results of the ELISA and the Raji-cell FAMA test with each other and with the standard FAMA test was found. Assuming that serum samples from patients IA and IIIA both contained low levels of IgG, then the FAMA test and the ELISA incorrectly scored IA and IIIA, respectively, as negative (Table 1). Correspondingly, both assays scored 98.7% sensitive and 100% specific. Shanley et al. (8) reported that the ELISA was 90% sensitive and 97% specific compared with the FAMA test. Two possible explanations for this difference in ELISA sensitivity compared with that of the FAMA test are (i) differences in the VZV antigen used for the ELISA and (ii) the fact that we did not heat-inactivate our sera for the ELISA as did Shanley et al. (8). An unexpected finding in this study was the development of significant (fourfold or more) antibody rises in some of the previously immune contacts. This observation suggests that subclinical VZV infection in immune individuals may be more common than previously anticipated. In general, the results of this study suggest that the FAMA test with Raji cells and the ELISA are both potentially suitable alternatives to the standard FAMA test for serodiagnosis of VZV infection.

ACKNOWLEDGMENTS

We thank Carol Cleghorn, Doris Vails, Anne Weinrod, and Ruth Pedrick for their excellent technical assistance and June Jackson and Sharon Johnson for manuscript preparation.

This study was supported in part by Public Health Service contract NO1-NS-9-2324 from the National Institute of Neurological and Communicative Disorders and Stroke.

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