Sensitive Solid-Phase Radioimmunoassay for Detection of Human Immunoglobulin G Antibodies to Varicella-Zoster Virus

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A sensitive solid-phase radioimmunoassay for detection of antibodies to varicella-zoster virus (VZV) is described. The antigen consisted of a sonically disrupted extract of VZV-infected human embryo cells. ¹²⁵I-labeled rabbit anti-human immunoglobulin G (IgG) specific for the Fc portion of human IgG was used to detect human IgG bound to viral antigen. With this technique, 193 human sera were evaluated for their IgG antibody titer against VZV. Subjects included 62 healthy adults, 33 young children (12 healthy), and 49 patients. Titers obtained by the radioimmunoassay were compared with those obtained by indirect fluorescence antibody staining of membrane antigen. The radioimmunoassay technique described gave titers approximately 5×10^4 times higher than those shown by indirect fluorescence. It can be used for routine diagnosis, but is especially suited to determining immune status to VZV, as defined by presence or absence of antibodies to the virus; for epidemiological studies; or for determining patients at risk who are exposed to the virus. No heterotypic titer rises to VZV were observed in sera with fourfold or greater rises to Epstein-Barr virus or cytomegalovirus. Sera of eight subjects with fourfold or greater titer rises to herpes simplex virus reacted in various ways: in six cases no significant change occurred in titer to VZV; one had a significant decrease in titer by the radioimmunoassay; and one had a significant increase. Possible reasons for these titer changes are discussed.

Routine diagnosis of varicella-zoster virus (VZV) infection and determination of immune status to the virus is currently carried out by the complement fixation (CF) technique (8, 31), immunofluorescence tests (8, 31, 33), or an immunoperoxidase technique (15).

The CF test for antibodies to VZV is too insensitive to be used for determination of immune status to VZV and is beset by problems of cross-reactions with herpes simplex viruses when used for diagnosis of current infections (8, 29, 31).

Several indirect fluorescent antibody methods have been described (31, 33, 35). They differ mainly in the type of antigen or antigen fixation procedure used. Some require living cell cultures, and others utilize stored antigen. All appear to be more sensitive than CF, and most are said not to suffer from cross-reactions with other herpesviruses (33, 35; see also 31). However, all require a fluorescence microscope, and evaluation of results is highly subjective.

Recently an immunoperoxidase technique for determination of neutralizing antibody and immunoglobulin G (IgG) antibody to VZV and

VZV membrane antigen has been described (15). The sensitivity of this technique is approximately equal to that of fluorescence techniques, and the technique has not shown cross-reactivity with other herpesviruses (15).

The most sensitive antibody assay technique is generally acknowledged to be radioimmunoassay (RIA). RIAs have been developed and described for a number of viral systems (2, 4, 7, 12-14, 18, 20, 21, 23, 24). An RIA adaptable for VZV has also been described (14). However, results of examination of sera of only one patient were described. Each serum dilution was tested in a glass vial bearing acetone-fixed cells infected with a cell-free VZV suspension.

In the present study we have developed a simple and sensitive RIA which utilizes antigen-coated polyvinyl microtiter plates as a solid phase. The results obtained by the RIA are compared with results obtained for the same sera by indirect fluorescent antibody staining of membrane antigens (IFAMA).

MATERIALS AND METHODS

Cells and media. Human embryonic fibroblasts

(HEF) were grown in medium M199 supplemented with 10% fetal bovine serum (FBS), 100 U of penicillin per ml, and 200 μ g of streptomycin per ml at pH 7.2. Batches of HEF were periodically tested and found to be free of mycoplasma.

Antigen preparation. Before infection, HEF cultures were split and allowed to grow to confluency. One of the resulting sister cultures was infected, and the other served as the source of control antigen. Cells were infected with VZV "Nurit" strain, isolated in 1973 from a chicken pox patient (25). Infected cultures were incubated at 35°C in M199 supplemented with 2% FBS at pH 8.0 until cytopathic effects were observed in 80 to 90% of the cells (usually 36 to 48 h after virus inoculation). Infected or uninfected (control) cells were briefly trypsinized with trypsin-ethylenediaminetetraacetic acid solution (0.25% trypsin; Biolab, Jerusalem) and were washed once in cold phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS) (pH 7.6), then suspended in PBS at a concentration of 5×10^6 per ml and sonically disrupted for 2 min in a Bransonic 12 sonifier. The sonic extract was centrifuged at $1400 \times g$ for 15 min, and the supernatant fluid, after protein determination by the method of Lowry et al. (26), was diluted to contain 0.3 mg of protein per ml (except where noted in Results) in PBS and stored at -20°C until needed.

In some experiments the use of trypsin was avoided. Cell monolayers in Roux bottles were washed with cold PBS. A 5-ml volume of PBS was then added to the monolayer, and the bottle containing the monolayer and PBS was frozen. Cells were removed from the monolayer by agitating the bottle when the PBS ice was just beginning to melt after removal from the freezer. The bottle contents were then sonically disrupted, and the sonic extract was clarified as described above. Both methods gave similar results and antigen of equivalent reactivity.

Human sera. Sera were obtained from presumably healthy second-year medical students and laboratory workers and from patients with clinical diagnosis of chicken pox or zoster or other viral diseases. Negative sera were obtained from young patients in our hospital and from previous epidemiological surveys (Central Virus Laboratory, Ministry of Health, Tel Aviv-Jaffa). Sera were heat inactivated for 30 min (56°C) before dilution with TFPBS (10% heat-inactivated FBS in PBS containing 0.05% Tween 20). Ten-fold serum dilutions were tested on both VZV-HEF antigen and HEF control antigen.

Iodine-labeled anti-immunoglobulin. The IgG fraction of rabbit anti-human IgG, Fc fragment specific, was purchased from Dako, Copenhagen. It was iodinated according to the method of Hunter and Greenwood (17) with chloramine T by Zvi Teitelbaum at the Nuclear Research Center, Beer-Sheva, Israel, to a specific activity of approximately 5 $\mu\text{Ci}/\mu\text{g}$ of protein. For use in the assay, the labeled anti-immunoglobulin was diluted in TFPBS to 2×10^5 to 5×10^5 cpm/ml.

RIA assay procedure. The RIA was carried out in U-shaped polyvinyl microtiter cups (Flow Laboratories, Irvine, Scotland). Antigen (0.025 ml of VZV-HEF or control HEF) was added to appropriate wells and allowed to dry overnight at room temperature.

The antigen-coated plates were washed once with PBS, then filled with PBS supplemented to 20% with heat-inactivated FBS and incubated for 1 h at 37°C. The plates were rinsed twice with PBS and dried. Then 0.025 ml of either the human serum dilutions to be tested or control diluent (TFPBS) was dropped into appropriate wells, and the plates were incubated at 37°C for 1 h. The plates were cooled, and after three rinses with cold PBS containing 0.05% Tween 20 (PBS-Tween), 0.025 ml of diluted ^{125}I -labeled goat anti-human IgG (Fc specific) was dropped into each well. After 1 h at 37°C the plates were again cooled, the labeled reagent was aspirated, and the plates were rinsed five times with cold PBS-Tween. When dry, the wells were clipped out, inserted into tubes, and counted for 4 min in a Packard Autogamma Spectrometer.

The following controls were included in each experiment: (i) serum diluent instead of human serum was added to at least six wells coated with VZV antigen and to six wells coated with control HEF antigen; (ii) a known positive serum and a known negative serum were included among the sera tested; (iii) all sera were tested on both VZV and HEF antigen.

IFAMA. The IFAMA technique was carried out as described previously (25). Briefly, infected or uninfected (control) cells suspended in PBS were dropped onto slides and allowed to dry. Twofold human serum dilutions in PBS were added and incubated at 36°C for 45 min, and the slides were washed in PBS. The cells were then treated for 30 min with fluorescein-conjugated goat anti-human IgG and washed in PBS. Slides were mounted in 10% glycerol-phosphate-buffered saline for examination. Antibody titers were expressed as the reciprocal of the last dilution giving a 2+ (out of 4) reaction, a distinct fluorescence ring around the surface of the infected cell.

RESULTS

Serum diluent. To test whether the type of serum diluent had any effect on the assay system, we made serial 10-fold dilutions of a positive and a negative serum in PBS, in PBS supplemented to 10% with heat-inactivated fetal bovine serum (FPBS), and in TFPBS. The diluted sera were then tested on VZV antigen and on control HEF antigen. The use of PBS alone as serum diluent resulted in erratic titration curves and unacceptably high background counts, especially on control antigen. The addition of FBS to a concentration of 10% resulted in reduction of this high background and increased reproducibility between duplicate determinations for each serum dilution tested. Additional supplementation of the diluent with Tween 20 to a concentration of 0.05% further reduced nonspecific reactions, lowering the negative serum curve on VZV and on control antigen and the positive serum curve on control antigen. The use of TFPBS as diluent also made possible the storage of serum dilutions at -20° for at least 3 months without loss in titer, probably because

FBS acts as a stabilizer.

Determination of serum titer. There is as yet no generally accepted formula for determination of serum titer by RIA; each investigator uses an arbitrary definition that gives him most consistent results (6, 14, 23, 27). The definition of titer may be based on either of two control values: the radioactive counts per minute (cpm) bound when each serum dilution is tested on control (nonviral) antigen, or the cpm bound when serum diluent is tested on viral antigen.

Thus a serum titer might be defined as the reciprocal of the last dilution at which the cpm bound to viral antigen is significantly higher than that bound by the same serum dilution on control antigen. "Significantly higher" may be defined as 2 or 3 standard deviations above the mean value bound with control antigen. However, such a definition is not practical, since it implies testing minimally three or four replicates of each serum dilution on viral and control antigen, thus limiting each assay to examination of a few sera and requiring correspondingly greater amounts of antigen and control antigen.

Alternatively, "significantly higher" may be defined as 2 times the cpm bound by each serum dilution on control antigen (14). If this definition of titer were to give consistently reproducible results for all or almost all sera, it might be the method of choice for titer determination (but see section on nonspecific control antigen reactivity, below).

A third possibility is that serum titer be defined as the last point at which the cpm bound to viral antigen is above a critical value with respect to the cpm bound to the antigen by serum diluent alone, with two stipulations: (i) that all serum dilutions have approximately the same protein content (insured by using a diluent with a relatively high protein content), and (ii) that the cpm bound to control antigen is lower than the critical value at the titer point.

Figure 1 shows titration curves for two sera tested on VZV-HEF and control HEF antigen. The reference line marks 2 times the counts bound by serum diluent alone on VZV-HEF antigen (average of at least six determinations). We define serum titer as the $\log_{10}$ reciprocal dilution at the intersection of the serum titration curve with this line. Using this definition we have determined titers for sera of 97% of the subjects we have tested.

Nonspecific control antigen reactivity. Some of the 193 sera we tested showed control antigen reactivity, defined as a control antigen serum titration curve lying in part above the reference line. This phenomenon has been previously noted in other RIA systems (14, 23).

FIG. 1. Determination of serum titer. Shown are titration curves of two sera on VZV antigen and control HEF antigen. Solid lines, solid symbols: Sera tested on VZV antigen. Dashed lines, open symbols: Sera tested on HEF control antigen. Reference line marks 2 times average background counts (serum diluent on VZV antigen). The titer of serum 785 (squares) is 6.4, and that of serum 414 (circles) is 8.0 reciprocal $\log_{10}$ units.

Generally the control antigen activity was limited to the first two serum dilutions and, in the case of most positive sera, did not affect titer determination, since most serum titers were between 4 and 8 reciprocal $\log_{10}$ units.

A serum was considered positive if its titration curve on viral antigen lay above the reference line for at least the first two dilutions (i.e., to a concentration of 1:100) and its titration curve on control antigen lay everywhere below that on viral antigen. In Fig. 2 are shown titration curves of two negative sera. Figure 2A illustrates a normal negative serum titration curve, and Fig. 2B shows a negative serum with control antigen reactivity tested in the same experiment.

In only a few cases was control antigen reactivity high enough to interfere with titer determination. Sera of 5 of the 144 subjects we tested were positive for VZV antibodies with respect to the criteria detailed above, but the control antigen serum titration curve intersected the viral antigen serum titration curve before the latter crossed the reference line. In these cases, the point of this intersection was determined to be the serum titer. However, when these sera were retested on a different antigen lot, three displayed reduced control antigen reactivity. This reduction suggests, perhaps, differences between different batches of HEF cells. It also may explain the relative lack of reproducibility which

we have found for serum titers determined by the 2-times-control antigen method when these sera have moderate to high control antigen reactivity and are tested on different lots of antigen.

Since sera with some degree of control antigen reactivity were found in all classes of subjects tested—children and adults, healthy and ill—we wished to investigate the problem further.

To determine whether these non-VZV-specific reactions could be ascribed to increased “stickiness” of some component of these sera (recognized by anti-human IgG) to protein in general, we further examined three sera which showed control antigen reactivity on two lots of antigen. We tested these sera on “antigen” consisting of bovine serum albumin at a concentration of 0.3 mg/ml, as well as on VZV-HEF and HEF control antigen at the same concentration. The titration curves obtained (Fig. 3) show that, whereas the interaction with control HEF cells was different

for each serum, the level of reactivity with bovine serum antigen was the same for all of them, indicating that the reactivity with control HEF antigen is probably a specific characteristic of the serum and the particular batch of HEF cells and is not due to indiscriminate sticking.

Determination of optimal antigen concentration. Several positive and negative sera were titrated in wells containing various concentrations of antigen (100, 200, 300, and 400 μ g/ml). Figure 4 shows an example of the titration curves obtained when one serum was tested on antigen at several different concentrations. At 100 μ g/ml there was greatest nonspecific binding (dashed lines) of antibody at high serum concentrations (1:10, 1:100). Maximum serum titer (sensitivity) was attained with an antigen concentration of 300 and 400 μ g/ml, and we chose to work with antigen at 300 μ g of protein per ml.

Reproducibility. One serum, tested in eight different experiments, had an average titer of 5.8 with a standard deviation of $\pm 0.35 \log_{10}$ units. The maximum titer difference found for a serum tested in separate experiments was 1.1 $\log_{10}$ units, although this was not a usual occurrence; Table 1 shows the results of testing two sera on different lots of antigen in different experiments. It can be seen that even on different lots of antigen serum titers were highly reproducible.

The maximum titer difference found for a serum tested in duplicate in the same experiment was 0.5 $\log_{10}$ units; most commonly, duplicate titer determinations had an intraexperimental variation of 0.0 to 0.2 $\log_{10}$ units.

Distribution of titers in a general population. A sample of 62 sera of a healthy adult population (medical students and laboratory personnel) were evaluated for titer to VZV by

FIG. 2. Titration curve of a normal negative serum (A) and a negative serum with relatively high control antigen reactivity (B). Solid and dashed lines and reference line as in Fig. 1.

FIG. 3. Comparison of non-VZV-specific reactivities of three sera. Solid lines, Sera tested on VZV antigen; dashed lines, sera tested on HEF control antigen; dotted lines, sera tested on bovine serum albumin (0.3 mg/ml). Reference line, see legend to Fig. 1.

FIG. 4. Serum titration curves showing effect of varying concentrations of antigen dried onto microtiter plates. Antigen protein concentration: ($\blacktriangle$) 100; ($\times$) 200; ($\bullet$) 300; ($\blacksquare$) 400 $\mu\text{g/ml}$. Solid lines, VZV antigen. Dashed lines, HEF control antigen.

TABLE 1. Titrations of two sera on different lots of antigen in different experiments

Serum	Expt	Antigen lot	cpm/well	Serum titer
601	6	PE-4	9,800	8.8
	8	S31.12	5,800	8.6
	16	S15.1	8,000	8.7
199 _{III}	9	FTS	6,500	7.5
	11	S15.1	10,000	7.6

the RIA method described and by the IFAMA technique. One serum was negative by RIA, and the remainder had titers ranging from 3.5 to 8.4 reciprocal $\log_{10}$ units (3.2×10^3 to 2.5×10^8). When titers were grouped in intervals of 1.5 logs, the distribution illustrated in Fig. 5 was obtained. For comparison, the distribution of titers obtained by IFAMA is also shown in Fig. 5. Three sera that were negative by IFAMA had titers of 3.8, 3.9, and 4.7 $\log_{10}$ units by RIA.

The absolute titers obtained by RIA were higher than those obtained by IFAMA. The average titer (geometric mean titer) for the population sample was 4.6 by IFAMA and 2.4×10^5 (5.39 $\log_{10}$ units) by RIA. If a comparison of these averages is taken as a measure of sensitivity, then the RIA is approximately 5×10^4 times more sensitive than IFAMA.

Negative sera. Because of the small number of seronegative individuals found in our survey of a healthy population, we tested 41 sera of 33 children between the ages of 8 months and 3 years. Of these children, 12 were healthy and 21 were hospitalized for various reasons. Eleven of the 12 healthy children were seronegative by RIA, as were 17 of the 21 hospitalized children. The five seropositive children (aged 12 to 24 months) had titers that were relatively low (between 2 and 3.6 reciprocal $\log_{10}$ units; i.e. titers between 100 and 8,000) compared to normal adult titers to VZV. Further studies are required

FIG. 5. Distribution of serum titers against VZV in a healthy adult population (ages 18 to 45). Titers were determined by IFAMA and RIA. Since RIA titers are determined to the nearest one-tenth log unit, they are grouped in intervals of 1.5 reciprocal log units for better graphic representation. The interval midpoints are noted on the abscissa. The IFAMA titers are reciprocal dilutions. For comparison note also characteristic titers of patients with VZV-associated illnesses, shown in Table 2.

to determine whether low titers are characteristic for this age group for children who have recovered from uncomplicated varicella. It may be that the higher titers found in adults are the result of repeated antigenic stimulation (25).

Diagnosis of current VZV infections. Figure 6 illustrates serum titration curves of paired sera from two patients, one ill with varicella and bronchiolitis (Fig. 6A) and one ill with herpes zoster (Fig. 6B). Both patients had diagnostic titer rises (varicella, 2.3 to 5.9 reciprocal $\log_{10}$ units; zoster, 5.3 to 7.0 reciprocal $\log_{10}$ units).

The results of examination of 17 sera of nine patients with VZV-associated illnesses by the RIA and IFAMA techniques are summarized in Table 2. Six patients had a fourfold or greater

titer rise by IFAMA and had a titer rise of greater than 1.0 $\log_{10}$ units (i.e., 10-fold or more) by RIA. Three patients had no titer rise (for two of them, only one serum sample was available), but their RIA titers to VZV (8.6 and 9.0) were above the titer range of the healthy population sample we had tested.

Twenty-seven sera of 12 patients with unrelated diseases were examined, and none showed significant VZV titer differences between acute and convalescent sera by RIA. Thus RIA seems to be as reliable an indicator of current VZV infection as IFAMA is.

It will be noted that in most patients "sero-conversion" (that is, conversion from seronegative to positive) as such was not found. This may be because sera from cases of uncomplicated varicella rarely come to the diagnostic laboratory. Acute sera are usually taken at the time complications develop from varicella or when reactivation occurs (zoster), and serum titers are often already in the range of normal serum titers (or higher). As indicated in Table 2, a few days may sometimes suffice to show a significant increase in titer. Diagnosis can then be made before 2 weeks have elapsed.

Cross-reactivity with other herpesviruses. Gross cross-reactivity of our VZV antigens with those of other herpesviruses was not seen among the sera we tested with moderate to high titers to other herpesviruses (Table 3).

In addition, paired sera from a patient with a significant titer rise to Epstein-Barr virus (EBV) viral capsid antigen (16) and from a patient with cytomegalovirus (CMV) mononucleosis diagnosed by IFAMA were tested for antibody titer to VZV by RIA. No significant change in RIA titer to VZV was observed for either patient (Table 4).

Because of several reports in the literature that titer rises against herpes simplex virus (HSV) antigens are sometimes accompanied by titer rises against crude CF VZV antigen (22, 29, 31), we examined a group of 18 sera from eight patients with fourfold or greater titer rises to HSV as determined by the IFAMA technique or, in one case, by CF. RIA VZV titers of one of these patients dropped significantly (Table 4), titers of six were unchanged or rose slightly, but not significantly, and the anti-VZV titer of one serum pair rose significantly. A history of this patient was unavailable, and no attempts at virus isolation were carried out. Therefore the possibility of double infection cannot be ruled out in this case.

DISCUSSION

In the present study we have developed an RIA for the detection of antibodies to VZV. The

FIG. 6. Diagnosis of VZV infections. (A) Sera of 3-month-old infant with varicella and bronchiolitis. Triangles, serum 385_I taken soon after onset of illness. Circles, serum 385_{II} drawn 22 days later. (B) Sera of 47-year-old female with zoster. Triangles, Serum 327_I drawn 2 days after onset of illness; circles, serum 327_{III} drawn 17 days later. Solid lines, dashed lines, and reference line, see legend to Fig. 1. Serum titers are: 385_I, 2.3; 385_{II}, 5.9; 327_I, 5.3; 327_{III}, 7.0 reciprocal log₁₀ units.

TABLE 2. VZV antibody titers in acute- and convalescent-phase sera from patients with varicella or zoster infections as determined by RIA and IFAMA

Patient no.	Serum no.	Patient age	Illness day ^a	Diagnosis	VZV titer	
					RIA ^b	IFAMA
1	385 _I	3 mo.	U	Varicella, bronchiolitis	2.3	≤2
	385 _{II}		+22		5.9	8
2	79 _I	8 mo.	U	Encephalitis following varicella	5.8	32
	79 _{II}		+14		7.0	128
3	401 _I	20 mo.	5	Cough, fever following varicella	4.9	<2
	401 _{II}		15		7.8	8
4	271 _I	24 yr.	3	Ramsay Hunt's syndrome	6.0	16
	271 _{II}		10		9.0	64
5	199 _I	32 yr.	2	Ramsay Hunt's syndrome	5.0	2
	199 _{II}		4		6.4	8
	199 _{III}		15		7.7	>48
6	327 _I	47 yr.	2	Ramsay Hunt's syndrome	5.3	4
	327 _{III}		19		7.0	16
7	368 _I	6.5 yr.	6	Varicella, facial paralysis	9.0	64
	368 _{II}		14		9.0	64
8	122	5 yr.	U	Necrotizing varicella	9.0	96
9	601 _I	20 yr.	4	Zoster	8.6	32

^a Days after onset of current illness. U, Unknown.

^b Titer in reciprocal log₁₀ units.

method is sensitive and rapid to perform. Using this method we have titrated the sera of 62 healthy adults. Positive serum titers ranged from 3.5 to 8.4 reciprocal log₁₀ units (3,200 to 2.5 × 10⁸). When the distribution of titers obtained by

RIA was compared with that obtained for the same population by IFAMA (Fig. 5), the RIA was on the average 50,000 times more sensitive than IFAMA.

However, possibly of more significance is the

clarity with which the RIA discriminates between positive and negative sera. The fact that healthy adult serum titers against VZV as deter-

TABLE 3. *Specificity of RIA for detection of antibodies to VZV*

Serum no.	Patient age	Titer to VZV		Titer ^a to		
		RIA ^b	IFAMA ^c	HSV	EBV	CMV
30	24 mo.	<2	<2		32	256
82	16 mo.	<2	<2	320		
86	18 mo.	<2	<2	320		
88	3 yr.	<2	<2	160	40	
99	18 mo.	<2	4	640		
176	12 mo.	<2	<2	<40		160

^a Titers determined by H. Haiken, Soroka Medical Center, Beer-Sheva, by an indirect peroxidase-conjugated antibody-membrane antigen technique (manuscript submitted for publication).

^b Reciprocal log₁₀ units.

^c Reciprocal dilution.

mined by IFAMA are relatively low may make it difficult in some cases to discern the difference between seronegativity and seropositivity. Indeed, three sera which appeared negative by IFAMA proved to be positive by RIA. With the RIA technique, no positive sera of healthy adults were found with titers below 3.5 log₁₀ units. The less commonly low-level titers are found in a population sample, the more certain is the discrimination between seropositive and seronegative individuals. This is important not only for epidemiological surveys but also in clinical situations where the presence or absence of antibodies to VZV, as an indication of previous infection, must be determined. Although infection with VZV generally results in mild disease, susceptible adults, immunocompromised children, or those with malignant disease risk severe illness if they contract varicella (9). In addition, the possibility that maternal-fetal infection with varicella has teratogenic effects has been dis-

TABLE 4. *VZV antibody titers of paired sera of patients with significant titer rises to other herpesviruses*

Patient no.	Serum no.	Patient age (yr)	Illness day ^a	Diagnosis	HV titer		VZV RIA titer ^b
					Virus	Titer ^c	
1	6768		3	Titer rise to EBV	EBV	10 ^d	4.6
	7344		130			160	4.4
2	198 _I	25	13	Mononucleosis	CMV	160	5.0
	198 _{III}		17			1,280	5.0
3	1047	40	7	HSV type 2	HSV	80	7.5
	1047 _I		35			320	6.0
4	2057	2	3	Fever, enlarged lymph nodes	HSV	80	<2
	2058		18			320	<2
5	785 _I	25	5	Encephalitis	HSV	80	7.0
	785 _{III}		14			320	7.3
6	1443	64	8	Encephalitis	HSV	80	4.0
	6954		18			640	4.2
	6955		32			1,280	4.0
7	1883 _{II}	50	10	Guillain-Barre syndrome, encephalitis	HSV	80	3.0
	1883 _{III}		25			640	3.2
	1883 _V		68			320	2.8
8	8865	62		Guillain-Barre syndrome	HSV	640	4.7
	8866					2,560	5.0
9	7144 _I	62	8	Viral encephalitis	HSV	80	4.0
	7144 _{II}		24			320	4.5
10	01379 _I	4	3	Bronchopneumonia	HSV	30 (CF)	4.0
	01379 _{II}		20			480	5.2

^a Days after onset of current illness.

^b Reciprocal log₁₀ dilutions.

^c Herpesvirus (HV) titer, determined by IFAMA except where noted.

^d Titer to viral capsid antigen determined by T. Gotlieb, Central Virology Laboratory, Tel Aviv-Jaffa, as described (16).

cussed (34), and some retrospective surveys have associated congenital varicella with subsequent development of childhood acute lymphatic leukemia (1, 5, 32). When the immunological status of such individuals is known, proper steps may be taken to prevent or modify varicella infection of susceptible individuals when they are exposed to the virus (3, 9, 10, 11, 28). The sensitivity of the RIA suggests that it has the potential of being able to detect lower levels of antibody than do other serological methods currently in use, and thus to determine more accurately those at risk. Confirmation of this potential awaits examination of several hundred low-titered or apparently negative sera and follow-up of the donors to determine incidence of subsequent varicella-associated illness.

The RIA technique described here appears to be suitable for routine diagnosis with paired serum samples. We believe that a difference of $1.0 \log_{10}$ units between titers of paired sera tested in the same experiment (a 10-fold titer rise) is indicative of recent varicella infection or reactivation (Table 2). No significant titer rise to varicella was found when paired sera of 12 patients ill with diseases other than those caused by herpesviruses were examined.

Ten sets of paired sera showing IFAMA titer rises to other members of the herpes group, EBV, CMV, and HSV, were also examined (Table 4). By this RIA method, no significant heterologous titer rises between VZV and EBV or CMV were detected. Also, we found sera with significant antibody titers to EBV, CMV, and HSV that were, nevertheless, negative to VZV by the RIA method (Table 3). These results agree with those of other workers, who find that cross-reactions between EBV or CMV and VZV are generally not a problem (30, 33, 35). The situation with regard to HSV is, however, more complex. In 1964 Kapsenberg (22) suggested the possibility that HSV and VZV might share common antigens. Ross et al. (29) and Schmidt et al. (31) have shown that, in a certain proportion of patients with HSV infections, heterologous CF antibody titer rises to VZV also occur. Schmidt et al. (31) showed that these heterologous rises were less frequently detected by fluorescence and neutralization tests than by CF. Although in the present study we found one instance (out of eight patients) of a significant heterologous titer rise to VZV by RIA, the remainder of our cases of herpes seroconversions showed either only slight (insignificant) titer rises to VZV, no change, or a significant drop in VZV titer (Table 4).

The reason for the heterologous titer rises being found in only a certain percentage of individuals with HSV infections has not been ad-

equately examined. If there is an absolute cross-reactivity between certain viral antigenic components, then one would expect to see the heterologous titer rise in almost all sera examined as well as some reactivity with VZV antigen for all sera with high titers to HSV. However, an additional possibility, which we have not seen seriously considered in the literature, is that the same stimulus that induces reactivation of one virus (perhaps a temporary drop in cell-mediated immunity) may induce reactivation of the second as well, either concurrently or subsequently. If concurrently, one would expect to see a concurrent rise in titer to both viruses. If subsequently, the titer against the first may be falling while the titer against the second is rising.

By using VZV subunit antigens or purified virus proteins as antigens with the sensitive RIA technique here described, it may be possible to determine to which viral components titer rises are occurring. Kalimo et al. (19) have initiated studies of this type with HSV subunit antigens.

Although we have found infected cell lysate antigen to be suitable for determination of anti-VZV serum titers in 97% of our subjects tested, the use of purified viral antigens may allow more accurate titer determination for sera that have unusually high control antigen reactivity and only slightly higher infected cell lysate antigen reactivity.

By using secondary antibody directed against the Fc portion of human IgG, we have probably minimized binding of the radiolabeled secondary antibody to human IgG nonspecifically bound to infected cell Fc receptors, if these are induced in VZV-infected cells. Although proof of this requires further experiments utilizing radiolabeled human IgG devoid of antibodies directed against VZV, the fact that our negative sera have identical titration curves on infected and noninfected cell lysates suggests that Fc receptors on infected cells are not a significant problem in this RIA.

The RIA described in this report may not supersede fluorescence or enzyme-linked assay techniques for routine clinical diagnosis when equipment for handling radioisotopes is not readily available. However, it is quite simple and seems to be the technique of choice for epidemiological surveys and for clearest differentiation of seropositive and seronegative individuals.

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