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Sensitive Enzyme Immunoassay for Detecting Immunoglobulin M Antibodies to Sindbis Virus and Further Evidence that Pogosta Disease Is Caused by a Western Equine Encephalitis Complex Virus

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An antibody capture enzyme immunoassay (EIA) was adapted for the detection of immunoglobulin M (IgM) antibody to Sindbis (SIN) virus. Sera from humans with a febrile illness characterized by rash and arthralgia in eastern Finland (Pogosta [POG] disease) and Sweden (Ockelbo disease) and from humans with western equine encephalitis (WEE) virus infection in the United States were tested for IgM antibodies by EIA. Seroconversions were documented in patients with POG disease and with WEE virus infections by using SIN virus as antigen and rabbit anti-SIN virus immunoglobulin; this confirms previous observations that POG disease is caused by a virus closely related to SIN virus and that IgM antibodies to WEE complex alphaviruses are not type specific. This IgM EIA provided a sensitive diagnostic and research tool applicable to epidemiologic problems posed by POG disease.

Epidemic and routine diagnostic experiences with arboviruses have stimulated the development of rapid procedures for virus isolation and identification, antigen detection, and antibody determinations. Antigen detection has been done principally by immunofluorescence assay (6) and enzyme immunoassays (EIA) (9, 13, 16). Burke et al. (3) and Monath et al. (14) have reported the successful use of EIA for detecting immunoglobulin M (IgM) antibodies in the diagnosis of infections caused by Japanese and St. Louis encephalitis viruses. Heinz et al. (8) reported the application of EIA in the diagnosis of tick-borne encephalitis, Jamnback et al. (10) have developed an EIA for detection of IgM antibodies to La Crosse virus, and Niklasson et al. (17) have reported the successful application of these tests for serodiagnosing infections caused by Rift Valley fever virus. By adapting the IgM capture EIA, it was possible to rapidly perform specific diagnoses with cerebrospinal fluids from encephalitis patients, with single serum samples collected in the acute phase of illness, and with paired sera collected at appropriate intervals from virus-infected individuals. Both polyclonal and monoclonal antibody preparations were used in these tests.

Extensive cross-reactivity can confound the interpretation of results of classic serologic tests such as hemagglutination inhibition (HI), complement fixation, and neutralization. The specificity of IgM antibody in virus infections has not been fully explored, but in one study with sera collected from humans after infections caused by St. Louis encephalitis virus, production of heterologous antibody to other flaviviruses occurred in about 28% of the patients (14). Early IgM antibody responses in infections caused by flaviviruses are without extensive cross-reactivity, even when tested with closely related viruses (19), but in superinfections of dengue viruses, IgM antibody responses are extensive and cross-reactivity is significant.

We have found few published reports of IgM antibody responses in human infections with group A (family *Togaviridae*, genus *Alphavirus*) viruses. Within this serogroup, six antigenic complexes are recognized (4). One of these, the western equine encephalitis (WEE) complex, includes WEE, Sindbis (SIN), Fort Morgan, Highlands J, Whataroa, and Kyzlagach viruses. Another virus, at present not included in this antigenic complex, causes a disease known as Ockelbo disease in east-central Sweden (18), Pogosta disease in eastern Finland (2), and Karelian fever in the western Soviet Union (12). This virus causes a disease characterized by fever, rash, and arthralgia in affected humans, and its epidemiology suggests transmission by an arthropod vector. Early serologic studies by one of us (M.B.-K.) indicated that patients with Pogosta or Ockelbo disease seroconverted to SIN virus in HI tests. Studies by L'vov et al. extended these findings to show that results of complement fixation tests with sera from patients with serologically confirmed cases of Karelian fever did not support the results of HI and neutralization tests with SIN virus (12). This indicated that Karelian fever is caused by a virus related, but not identical, to prototype SIN virus. A candidate virus has been isolated recently (11). Ockelbo virus has been isolated from mosquitoes and possesses biologic and antigenic characteristics similar but not identical to prototype SIN virus (15). Thus, the etiologic agent of this disease is a member of the WEE complex of group A arboviruses.

This report presents results of EIA for IgM antibody in sera from humans in Finland with clinically diagnosed Pogosta disease. The results unequivocally demonstrate that Pogosta disease is caused by a virus related to SIN virus. IgM antibodies in humans with Pogosta disease in Finland or with WEE virus in the United States are shown to react with SIN virus.

MATERIALS AND METHODS

Viruses. Prototype SIN virus strain AR 339, kindly provided by J. M. Dalrymple, U.S. Army Medical Research

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TABLE 1. HI, IgM, and IgG enzyme immunoassay titers of 77 sera from 30 individuals with clinically diagnosed Pogosta disease in Finland by days after onset

Patient no.	Days after onset	Titer with SIN virus antigen:		
		HI	IgM	IgG
1	2	—[a]	—	—
	12	40	1,600	80
2	1	—	—	—
	12	160	≥25,600	160
3	1	—	—	—
	12	160	3,200	640
4	1	—	—	—
	15	160	1,600	160
5	1	—	—	—
	16	40	1,600	320
6	0	—	—	—
	10	80	800	320
7	1	—	200	—
	14	40	1,600	320
8	1	—	—	—
	12	40	3,200	320
9	1	—	—	—
	14	160	3,200	160
10	14	80	400	640
	28	80	400	1,280
11	4	—	400	—
	18	80	6,400	1,280
12	2	—	—	—
	15	40	800	80
13	1	—	400	—
	48	40	400	160
14	4	20	25,600	—
	18	40	6,400	80
	71	80	—	—
15	5	10	—	—
	26	80	6,400	40
	75	80	1,600	80
16	1	—	—	—
	26	40	400	160
	78	80	—	320
17	1	—	—	—
	28	40	—	—
	85	80	1,600	80
18	2	—	—	—
	17	80	25,600	80
	58	80	1,600	80
19	0	—	—	—
	24	160	25,600	1,280
	81	160	6,400	640
20	7	20	25,600	1,280
	22	40	25,600	1,280
	79	80	400	1,280
21	2	—	—	—
	15	80	1,600	80
	74	80	—	—
22	3	—	6,400	80
	15	40	25,600	1,280
	80	80	1,600	320
23	1	—	—	—
	17	40	25,600	1,280
	71	80	—	320
24	2	—	—	—
	17	40	25,600	1640
	82	40	1,600	320
25	2	—	—	—
	20	20	25,600	320
	77	80	1,600	640
26	15	10	6,400	—
	34	160	25,600	1,280
	95	160	400	320
27	1	—	—	—
	19	40	25,600	640
	70	80	25,600	320
28	27	—	1,600	160
	64	—	—	80
	111	—	—	40
29	6	—	800	—
	21	—	800	—
	63	—	200	—
30	6	—	—	80
	20	—	—	80
	50	—	—	—

[a] —, Titer of <10 by HI and <100 and <40 by E1A with IgM and IgG, respectively.

Institute for Infectious Diseases, Fort Detrick, Frederick, Md., was used to prepare antisera in laboratory animals and to develop EIA. Ockelbo virus strain Edsbyn 5/82, kindly provided by B. Niklasson, Department of Virology, National Bacteriological Laboratory, Stockholm, Sweden, was used to prepare antigens. WEE virus strain Fleming was obtained from the reference collection of arboviruses maintained at the Centers for Disease Control, Fort Collins, Colo., and used for antigen preparation.

Antigens, secondary antibody, labeled antibody, and substrate. After preliminary titrations of all reagents with gradient-purified SIN virus, infected cell lysate or partially purified antigens of SIN, Ockelbo, and WEE viruses were used in subsequent tests. For preparation of both of these antigens, Vero cells grown in roller bottles were infected with approximately 1,000 median tissue culture doses of virus, and both cells and supernatant fluids were harvested when cytopathic effects were 3+ to 4+ (about 72 h after infection). After light centrifugation (2,500 rpm, 10 min), cell debris and supernatant fluids were separated. Cells from three to five roller bottles were taken up in a total of 1 ml of phosphate-

buffered saline and sonicated. This was then recentrifuged (2,500 rpm, 30 min), and the supernatant was diluted 1:10 for use as cell lysate antigen.

For preparation of partially purified antigens, supernatant fluids from infected cells grown in roller bottles were centrifuged in 30-ml quantities at 20,000 rpm for 3 h. Supernatant fluids were discarded, and all pellets were taken up in a total of 1 ml of phosphate buffer (pH 6.8) and diluted 1:10. Although both cell lysate and partially purified antigens gave similar results, the latter was used for all tests reported here because, in titrations in which a single sandwich EIA was used, partially purified viruses produced titers of 1:100 to 1:1,000 (10- to 100-fold higher than those produced by cell lysate antigen) and consistently produced antibody titers at least 10-fold higher than those produced by cell lysate antigens.

Rabbits were used to produce virus antibody. The immunizing regimen was modified from that of Halonen et al. (7). Ten-month-old, 3.5-kg New Zealand rabbits were given four doses of gradient-purified SIN virus diluted in phosphate-buffered saline. The first three doses were given intradermally in 0.5-ml volumes containing equal quantities of 175 to 250 μg of viral protein (Bio-Rad Laboratories, Richmond, Calif.) in phosphate-buffered saline and Freund adjuvant. Sera from these animals were tested by direct EIA, and a fourth dose (200 μg) was administered intravenously without adjuvant. Doses were given on days 0, 28, 50, and 72, and the rabbits were exsanguinated on day 78. Rabbit anti-SIN virus immunoglobulin fractions were prepared by precipitation of sera with 18% (wt/vol) Na_2SO_4 followed by Sephadex G-25 chromatography. These immunoglobulin preparations were stored at −70°C until used for EIA.

Commercially available horseradish peroxidase-conjugated swine antibodies against rabbit IgG (Orion Diagnostica, Helsinki, Finland) were used to detect rabbit anti-SIN virus immunoglobulin. The substrate solution, prepared fresh just before use, consisted of 45 mg of *o*-phenylenediamine dihydrochloride (Eastman Kodak Co., Rochester, N.Y.) in 15 ml of 0.1 M citrate–Na_2HPO_4 buffer (pH 5.5) and 10 μl 30% hydrogen peroxide.

IgM antibody determinations by EIA. A double-sandwich technique was used to determine IgM antibody. Plates were consecutively treated with (i) goat anti-human μ-chain antibody, (ii) human test serum, (iii) antigen, (iv) rabbit anti-SIN virus immunoglobulin, (v) anti-rabbit swine IgG labeled with horseradish peroxidase, and (vi) substrate. Linbro (Flow Laboratories, Inc., McLean, Va.) microtiter plates, recommended for EIA, were used as the solid phase. IgG fraction goat anti-human μ-chain antibody (Cappel Laboratories, West Chester, Pa.) was diluted in carbonate buffer (3), pH 9.6, for coating the wells. Diluent for antigens and all antibodies except the coating (capture) antibody was phosphate-buffered saline (pH 7.4) containing 5% heat-inactivated fetal bovine serum, 0.5% Tween 20, and 40 μg of merthiolate per ml.

After each incubation, the plates were washed three times with phosphate-buffered saline containing 0.1% Tween by use of a semiautomatic device (ImmunoWash; Nunc, Roskilde, Denmark). The volume of all reagents was 100 μl.

Capture antibody was titrated with pretitrated sera from humans with IgM antibody to rubella virus. For IgM antibody tests with sera from patients, a final dilution of 1:600 (0.58 μg per well) in coating buffer was used to coat the plates. After overnight incubation at room temperature, either a 1:50 or a 1:100 screening dilution or one of a series of dilutions was dispensed into the appropriate wells. After 1 h of incubation at 37°C, a pretitrated 1:1,000 dilution of partially purified virus was added and the plates were incubated for 18 h at 4°C. Rabbit Anti-SIN virus immunoglobulin was then added, the plates were again incubated at 37°C for 1 h, and swine anti-rabbit IgG labeled with horseradish peroxidase was added at 1:2,000. After further incubation for 1 h at 37°C, substrate was added and the plates were incubated at room temperature in the dark until a color change was apparent, usually 10 to 15 min. The reaction was stopped with 1 N HCl, and optical densities were measured at 492 nm by use of a Multiskan Analyzer (Eflab OY, Helsinki, Finland) programmed by a Hewlett-Packard computer. The ratios of the absorbance values of given dilutions of positive test sera from patients with and without antigen to that of a negative control serum were calculated; positive/negative ratios of ≥2.0 were considered positive. To determine acceptable absorbance values for negative sera, 100 sera from humans of both sexes and all age groups were tested by EIA with purified and partially purified SIN virus. These sera had been previously shown to lack HI antibody to SIN virus (titers of <1:10). No differences between any of the groups or between sexes were noted insofar as nonspecific reactivity was concerned. Except for the antigens, EIA tests for IgM antibody in sera from humans with Ockelbo and SIN viruses were performed by the same procedure as for detecting IgM antibody to SIN virus.

For IgG assays, 500 ng of purified SIN virus antigen was added in coating buffer to each well of a plate. After the plates were washed, dilutions of patient sera, beginning at

TABLE 2. HI, IgM, and IgG EIA titers of 21 sera from 10 individuals with clinically diagnosed Pogosta disease in Finland, dates of onset unknown

Patient no.	Phase	Titer with SIN virus antigen:		
		HI	IgM	IgG
31	Acute	20	102,400	1,280
	Convalescent	80	102,400	1,280
	Late convalescent	320	6,400	320
32	Acute	—[a]	—	—
	Convalescent	40	100	160
33	Acute	—	400	—
	Convalescent	20	400	160
34	Acute	20	400	—
	Convalescent	160	3,200	640
35	Acute	—	—	—
	Convalescent	20	1,600	2,560
36	Acute	80	200	320
	Convalescent	160	3,200	2,560
37	Acute	20	1,600	320
	Convalescent	80	800	1,280
38	Acute	—	—	—
	Convalescent	—	≥1,600	80
39	Acute	—	800	320
	Convalescent	—	100	160
40	Acute	—	3,200	80
	Convalescent	—	800	320

[a] —, Titer of <10, <100, and <40 with HI, IgM, and IgG, respectively.

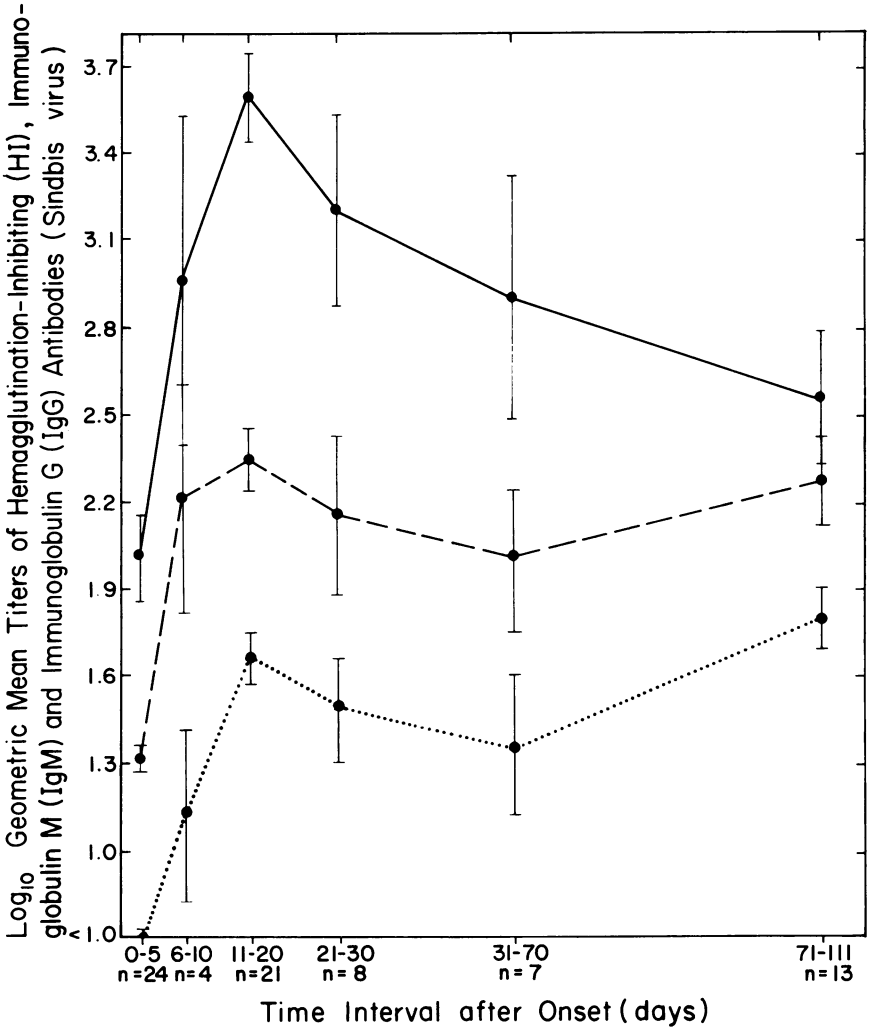

FIG. 1. Relationship of IgM (——), IgG (– – –), and HI (. . .) antibody titers to time interval after onset of illness in 30 individuals with Pogosta disease.

1:40, were added, plates were incubated overnight at 37°C and then washed again, and horseradish peroxidase-conjugated swine anti-human IgG (1:2,000) (Orion Diagnostica) was added. After further incubation at 37°C for 1 h, substrate was added and the optical density was read as outlined above. For HI tests, the method of Clarke and Casals (5) was used.

Clinical specimens. Ninety-eight serum samples were selected from among specimens collected from more than 400 individuals with Pogosta disease (exanthematous arthralgia) acquired during epidemics of that disease in Ilomantsi, eastern Finland, in 1974, 1981, and 1983. Of the 40 individuals tested, 30 had been shown to have increases in antibody titers of fourfold or greater between specimens collected in the acute and convalescent phases of illness (M.B.-K., unpublished data). In sera from the remaining 10 individuals, either HI antibody was absent (<10) or antibody was present but not rising significantly enough (i.e., <10 to 20; 80 to 80; 80 to 160) to consider them as serologically confirmed cases. The 30 individuals with significantly rising HI antibodies therefore represented most of the original 400 cases, whereas the remaining 10 were selected and considered problem cases.

Sera from humans with infections caused by Ockelbo virus in Sweden in 1982 were provided by B. Niklasson, State Bacteriological Laboratory, Stockholm, Sweden. Sera from humans infected with WEE virus in 1983 in Minnesota were from the diagnostic collection maintained at the Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins, Colo.

RESULTS

Results of HI, IgM EIA, and IgG EIA tests are shown in Table 1 (onsets known, cases 1 through 30) and Table 2 (onsets not known, cases 31 through 40), and summarized in Fig. 1. IgM antibody to SIN virus was detected as early as 1 day after reported onset of illness in 3 of 12 patients. Of 27 patients, 7 had IgM antibody within the first week after onset, but only 3 had HI antibody, and 3 had IgG antibody. Since the sera to be tested were selected principally on the basis of HI antibody titer rises with SIN virus, there is a bias toward HI positivity. Nevertheless, of 38 persons with clinical Pogosta disease (Tables 1 and 2) and at least fourfold increases or decreases in HI, IgM, or IgG titers to SIN virus, none were positive by HI alone, 3 (7.9%) were positive by IgM alone, 1 (2.6%) was positive by IgG alone, 8 (21.1%)

TABLE 3. Summary of titer changes in humans with clinically diagnosed Pogosta disease, as determined by EIA and HI tests with SINdbis virus as antigen

Result	No. (%) of changes in titer by:		
	EIA		HI
	IgM	IgG	
Increase[a]	17 (42.5)	22 (55)	30 (75)
Decrease[a]	7 (17.5)	2 (5.0)	0
Both[b]	10 (25)	3 (7.5)	0
Stable titer	5 (12.5)	12 (30)	4 (10)
No antibody	1 (2.5)	1 (2.5)	6 (15)

[a] Rise or fall of fourfold or greater in titer between paired or three sera.
[b] Rise and subsequent fall of fourfold or greater in titer between three sera.

were positive by HI and IgM, 3 (7.9%) were positive by HI and IgG, 4 (10.5%) were positive by IgM and IgG, and 19 (50%) were positive by all three. Of 40 patients, 34 had a fourfold rise or fall in IgM antibody titer; 5 of the other 6 patients had detectable IgM antibody; 27 of 40 (67.5%) patients had either a rise or a fall of fourfold in IgG antibody titer, and 12 of the remaining 13 patients had detectable IgG antibody (Table 3).

Sera from five patients with Ockelbo disease were tested for IgM and IgG antibodies by EIA with SIN virus, and the results were compared with neutralization data (courtesy of B. Niklasson) (Table 4). All sera without detectable antibody to Ockelbo virus were likewise without antibody to SIN virus. Conversely, sera with Ockelbo virus antibody had titers that were essentially identical to those of SIN virus antibody by neutralization and EIA.

Twenty-one serum samples from seven patients with WEE virus infections were tested for IgM and IgG antibody by EIA with SIN virus (Table 5). Patients 48, 49, 50, and 51 were serologically confirmed by rises of fourfold or greater in antibody titer to WEE virus. Patients 48, 50, and 51 had IgM antibody; patients 48, 49, and 51 had IgG antibody to SIN virus, although titers of SIN virus (heterologous) antigen were lower than those of WEE virus (homologous) antigen; and patient 46 was negative in all but HI tests. Moreover, seroconversion was detected by use of SIN virus for IgM (patient 48) and IgG (patient 51) EIA. In addition, acute- and convalescent-phase sera from patients 47 and 52 had IgG titers of 1:80. Partially purified SIN and Ockelbo viruses were used for EIA comparisons of their sensitivities in detecting IgM antibody in 24 serum samples from 13 patients with serologically confirmed Pogosta or Ockelbo disease (data not shown). None of 15 samples collected during the acute phase of illness and without detectable IgM antibody (<1:100) to SIN virus had IgM antibody to Ockelbo virus, but all of six convalescent-phase sera had equal titers (1:400 and 1:12,800) with both antigens. Three other convalescent-phase sera had IgM titers of at least fourfold higher with Ockelbo virus than with SIN virus.

TABLE 4. Results of plaque reduction neutralization and EIA for IgM and IgG antibodies in sera from five individuals with Ockelbo disease

Patient no.	Titer in sera from patients in[a]:								
	Acute phase				Convalescent phase				
	N		IgM (SIN)	IgG (SIN)	N		IgM (OCK)	IgM (SIN)	IgG (SIN)
	SIN	OCK			SIN	OCK			
41	<10	<10	<100	<40	40	20	3,200	3,200	1,280
42	<10	<10	<100	<40	40	80	25,600	12,800	1,280
43	NT	NT	NT	NT	160	80	<40	<100	320
44	NT	NT	NT	NT	80	40	800	400	320
45	NT	NT	NT	NT	40	40	12,800	12,800	2,560

[a] OCK, Ockelbo disease virus; NT, not tested.

TABLE 5. HI, complement fixation, and EIA for IgM and IgG antibodies to SIN and WEE viruses[a]

Patient no.	Age/ Sex	Days after onset	Titer of following virus[b]:					
			WEE				SIN (EIA)	
			HI	CF	EIA			
					IgM	IgG	IgM	IgG
46	45/M	3	40	—	—	—	—	—
		17	40	—	—	—	—	—
47	17/F	2	40	—	—	—	—	80
		35	80	16	—	—	—	80
		52	80	16	—	—	—	—
48	22/M	4	10	—	—	—	—	—
		13	160	—	>2,560	—	400	—
		15	320	—	>2,560	—	400	—
		23	160	—	>2,560	—	200	—
		25	320	—	>2,560	—	200	—
		32	320	8	>2,560	—	200	80
49	10/M	5	40	—	>160	—	—	—
		25	160	32	>160	>2,560	—	80
50	6/M	3	—	—	—	—	—	—
		8	—	—	—	—	—	—
		18	40	—	—	—	—	—
		30	320	16	—	—	100	—
51	15/M	4	—	—	—	—	—	—
		22	320	32	>160	>2,560	100	320
52	40/M	50	80	8	—	—	—	80
		85	80	8	—	—	—	80

[a] Sera were obtained form seven patients infected with WEE virus in Minnesota in 1983.
[b] —, titer of <10 by HI, <8 by complement fixation (CF), and <40 by EIA for WEE virus IgM or IgG antibody or SIN virus IgG antibody and <100 by EIA for SIN virus IgM antibody.

In an attempt to determine the extent of cross-reactivity of IgM antibody from patients with Pogosta disease and WEE virus infections, purified SIN virus was titrated by EIA with patient serum or anti-SIN virus guinea pig immunoglobulin for antigen capture and anti-SIN virus rabbit immunoglobulin or patient serum as secondary antibody. Serologic testing of a serum sample from a patient with Pogosta disease detected as little as 10 ng of SIN virus, whereas testing of serum from a patient with WEE virus infection with a similar IgM titer (WEE virus antigen) detected only 100 ng of SIN virus.

When anti-SIN virus guinea pig immunoglobulin was used for capture, sera from patients with Pogosta disease and WEE virus infections detected 0.1 ng of SIN virus, but serum from the patient with Pogosta disease had a higher titer (≥1:800 versus 1:200).

Carrying this one step further, we performed an EIA with IgM antibody from a patient with Pogosta disease, partially purified Ockelbo and SIN viruses, and rabbit anti-SIN virus immunoglobulin. This serum was found to have an IgM titer of 1:25,600 with Ockelbo virus and 1:1,600 with SIN virus.

DISCUSSION

The sensitive EIA described here is useful in detecting and quantifying IgM antibodies to SIN virus or Ockelbo virus in sera from patients with Pogosta disease. This test is more sensitive and easier to perform than the HI test and has the

advantage of not requiring pretreatment of the serum sample before testing. Since IgM antibodies appear early and decline rapidly in the course of infection, IgM antibody tests have certain advantages over HI and neutralization tests in diagnoses of recent infections and for serologic surveys in immediate postepidemic situations. Under the latter circumstances, testing for IgM antibody in combination with tests for HI or neutralizing, and possibly complement-fixing, antibody to Ockelbo virus is suggested. All but one of the 40 individuals tested had IgM antibody, so that even when seroconversion could not be documented, there was still evidence for recent infection in 97.5% of all patients. The EIA for detecting IgG antibody was approximately as sensitive as HI for diagnosis. The persistence of both HI and IgG antibodies (Fig. 1), however, contraindicates their usefulness in diagnostic tests on single serum samples.

Partial purification of the antigens used for the EIA tests reported here provides a relatively simply prepared reagent with significant potency. Infection of two or three roller bottles of Vero cells provides sufficient antigen for endpoint EIA titrations of IgM antibody in at least 2,000 serum samples, and preparation of cell lysate antigen from the residual cell debris from these same cells provides additional, if somewhat less potent, antigen.

That SIN virus is useful in detecting antibody in patients with Pogosta or Ockelbo disease is not in itself a new finding, since it was SIN virus with which the disease was initially serodiagnosed (2, 18). However, (i) the use of SIN and Ockelbo viruses for detecting IgM antibody in patients with Ockelbo disease (ii) evidence that IgM antibody reactive with SIN virus may be found in patients with WEE virus infections, and (iii) the successful use of various combinations of serum samples from patients with Pogosta disease and WEE virus infections for detecting SIN virus by antigen detection EIA together provide evidence that Pogosta and Ockelbo diseases have the same or very closely related etiologic agents and that these viruses are members of the WEE antigenic complex of group A arboviruses. This may be a basis for a portion of the antigenic cross-reactivity. Cross-reactions between Ross River and Getah viruses, also group A arboviruses but not belonging to the WEE complex, have been detected by immunofluorescence tests with IgM antibodies in sera from humans with Ross River virus infections (1).

The similarity of IgM antibody titers to SIN and Ockelbo viruses in patients with Ockelbo disease supports the findings of Niklasson et al. (16) that antibody in experimentally infected mice hyperimmunized with Ockelbo virus is of high neutralizing titer to either virus and that, in mice similarly immunized with SIN virus, neutralizing antibody to the homologous virus has a much higher titer than does antibody to the heterologous virus. This is probably the case with humans as well. It is fortunate that the one-way cross-reactivity is in this direction, for otherwise the serodiagnosis of Ockelbo and Pogosta disease, as well as Karelian fever, would probably have been delayed until Ockelbo virus was isolated.

That sera from patients with Ockelbo and Pogosta disease have IgM antibody titers to Ockelbo virus equal to or higher than those to SIN virus provides further evidence that both diseases share a similar etiology and that the IgM antibody assay used in these tests is both sensitive and reasonably specific. The isolation of Pogosta disease virus from patients will allow the necessary comparisons of Ockelbo and Pogosta disease viruses. Finally, the data suggest that antigen of Ockelbo virus, not SIN virus, be used in serologic tests for antibody in patients with Ockelbo disease.

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