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Serodiagnosis of Respiratory Syncytial Virus Infections in Infants and Young Children by the Immunoperoxidase Technique

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The immunoperoxidase antibody (IPA) technique was used to develop two new tests for serodiagnosis of respiratory syncytial virus infections: a microneutralization test based on the reduction of the number of infected cells and an IPA test for determination of virus-specific immunoglobulin G (IgG). Neutralizing antibody was determined both in the presence and absence of complement. In a group of 24 infants and young children, ages <1 to 36 months, with acute respiratory syncytial virus infection, serodiagnosis was made by the IPA-IgG test in 20 cases, by neutralization test with addition of complement in 19 cases, and by neutralization test without addition of complement in 17 cases. Complement fixation detected only 12 cases of infection. All four cases not serologically diagnosed were infants less than 1 month old. Neutralization test antibody titers in the presence of complement were usually 4- to 16-fold higher than titers obtained without addition of complement. Both IPA-IgG and neutralization test (in the presence of complement) appear very efficient in serologically detecting respiratory syncytial virus infections in infants older than 1 month and give rapid results (IPA-IgG takes 2 h to complete, and the neutralization test takes 24 h). However, IPA-IgG is simpler to perform.

Serodiagnosis of respiratory syncytial (RS) virus infections has usually been accomplished by complement fixation (CF) and neutralization tests (3, 7). However, both tests have been shown to be inefficient in detecting significant antibody increases in young infants during convalescence from respiratory disease caused by RS virus (3, 9, 12). This is thought to be due to immunological immaturity of infants or to impairment of the immune response caused by the presence of maternal antibodies (8, 9, 11). Recently, an enzyme-linked immunosorbent assay test (10) was proposed as a very efficient tool for detection of RS virus infections in infants 1 to 3 months old.

In the present report we present a new neutralization technique based on the reduction of RS virus-infected cells, as detected by the immunoperoxidase antibody (IPA) technique, which is completed in 24 h, and a new IPA test for RS virus-specific immunoglobulin G (IgG) for serological diagnosis of RS virus infections in infants and young children. In addition, the neutralization technique was performed in the presence of complement, since neutralization has been found to be more sensitive in the presence of complement (1, 6). Both neutralization and IPA-IgG tests were capable of detecting a significant rise in RS virus antibody in all cases of acute respiratory infections tested, except for infants in the first month of life.

MATERIALS AND METHODS

Cell cultures. HEp-2 cell cultures, originally obtained from ATCC (Rockville, Md.) at passage 365 to 375, and human embryonic lung fibroblasts (HELF) cell cultures at passage 20 to 30 were employed. For both types of cell cultures, Eagle minimal essential medium (E-MEM) supplemented with 10% fetal calf serum (FCS) was used as growth medium and E-MEM supplemented with 2% FCS was used as maintenance medium. Cells were grown for neutralization and IPA-IgG tests in tissue culture microplates (Falcon, Oxnard, Calif.) at 37°C in a humidified atmosphere of 5% CO_2 in air.

Virus. The Long strain of RS virus originally obtained from ATCC (Rockville, Md.) was used. Stock virus was prepared by infecting HEp-2 monolayer cell cultures in 150-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) with approximately 10^6 plaque-forming units. After 2 h of adsorption, maintenance medium (E-MEM supplemented with 1% chicken serum) was added, and incubation continued at 37°C. When 80 to 90% of the monolayer showed cytopathic effect, chicken serum was added to the medium to the final concentration of 5%. Cells were scraped into culture fluid and the material was stored frozen at −80°C. After various periods of storage, culture fluids were thawed and clarified by centrifugation at 200 × *g* for 10 min. The supernatant fluid contained between

10^5 and 10^6 infectious units of virus per 0.05 ml.

IPSCA. For the immunoperoxidase-stained cell assay (IPSCA), after removal of maintenance medium and three washings with Hanks balanced salt solution, replicate cell cultures prepared in flat-bottomed tissue culture microtiter plates were inoculated with 0.05 ml of cell-free virus suspension. The inoculum was aspirated after 1 h of adsorption at 37°C in a 5% CO_2-air atmosphere, and the cultures were washed once with approximately 0.2 ml of Hanks balanced salt solution and maintained with E-MEM containing 0.5% FCS (lots previously shown to be free of inhibiting activity to RS virus). Between 24 and 48 h after infection, the cultures were chilled, fixed in cold absolute ethanol, held at room temperature for 20 min, and then stained immediately or stored at −80°C. Fixed monolayers were reacted for 60 min at 37°C with RS virus immune horse serum (Flow Laboratories, Irvine, Scotland) previously absorbed with fixed human cells and diluted (usually 1:25 to 1:50) to give optimal staining. Negative controls consisted of uninfected cell cultures treated in the same manner. The cultures were washed three times with phosphate-buffered saline and reacted with peroxidase-conjugated goat anti-horse IgG (Cappel Laboratories, Cochranville, Pa.) for 1 h at 37°C. Three additional washings in phosphate-buffered saline removed excess conjugate. The diaminobenzidine-H_2O_2 color-developing system was used (5). Immunoperoxidase-stained cells were easily counted at 100× with an inverted microscope.

Neutralization test. Serial twofold dilutions of patients sera (inactivated at 56°C for 30 min) in E-MEM containing 0.5% FCS were mixed with equal volumes of virus suspension containing 30 to 60 infectious units of virus per 0.05 ml (as determined by IPSCA). Virus controls contained equal volumes of medium and virus suspension. Each serum was tested in duplicate in the presence and absence of complement. When the neutralizing activity of a serum was tested in the presence of complement, one half of the total volume of the mixture consisted of a serum dilution, one fourth consisted of virus suspension (diluted to contain 60 to 120 infectious units of virus per 0.05 ml) and one fourth consisted of guinea pig complement (Cordis Laboratories, Miami, Fla.) diluted to contain 50 50% hemolytic complement units in 0.4 ml, according to the laboratory branch CF test (2). Virus control mixtures in the presence of complement contained half a volume of medium and half of equal parts of virus suspension and complement. The mixtures were incubated at 37°C for 60 min and then transferred (0.05/well) onto monolayers of HELF microplate cell cultures for IPSCA. After 1 h of adsorption at 37°C in a 5% CO_2-air atmosphere, cell monolayers were washed with Hanks balanced salt solution and maintained with E-MEM supplemented with 0.5% FCS. The staining reaction was routinely done 24 h after inoculation of cell cultures. The highest serum dilution neutralizing 50% or more of the virus was considered to be the antibody endpoint. Duplicate antibody determinations gave identical results in about 95% of the cases. In the other cases, the variation was within a twofold dilution. HELF were preferred over HEp-2 cell cultures because of easier detection of RS virus-infected cells and absence of nonspecific background staining.

IPA test for determination of RS virus-specific IgG antibody. Microplate cell cultures for determination of RS virus-specific IgG in patients' sera were prepared as described for IPSCA. Confluent monolayers were infected with virus suspension diluted to contain 100 to 300 infectious units of virus per 0.05 ml. The inoculum was 0.05 ml/well. After 24 to 48 h of incubation at 37°C, cells were fixed as indicated above and stored at −80°C. A peroxidase-conjugated IgG fraction of goat anti-human IgG (gamma-chain specific) was commercially obtained (Cappel Laboratories, Cochranville, Pa.). The determination of the optimal conjugate dilution and the application of the sera and conjugate for RS virus IgG detection followed the same procedures described for determination of IgG specific for other viruses (4, 5). Briefly, twofold dilutions of test serum starting at 1:16 were layered onto cell cultures and incubated at 37°C for 60 min. After three washings with phosphate-buffered saline, the optimal dilution of the conjugate was added, and incubation was continued for another 60 min at 37°C. After three additional washings, virus-antibody reaction was detected histochemically with the diaminobenzidine-H_2O_2 color developing system (5). HEp-2 cell cultures gave problems of nonspecific staining with low serum dilutions. HELF cell cultures, which showed complete absence of background staining, were more advantageous and reliable.

CF test. The CF test was performed by the laboratory branch CF procedure (2) with use of RS virus-positive and RS virus-negative antigen and reference sera (Microbiological Associates, Walkersville, Md.). In all tests two units of antigen were routinely employed (3).

Isolation and identification of RS virus. Isolation of RS virus was routinely performed in HEp-2, MRC-5 (Flow Laboratories, Irvine, Scotland) or HELF cells, or both, and primary African green monkey kidney cell cultures, and virus was identified by means of an IPA technique identical to that reported for RS virus-infected cell detection in IPSCA. Specimens for virus isolation were throat swabs.

Serum specimens. Acute- and sequential convalescent-phase sera (four sera per patient) were obtained from patients admitted to the Pediatric Clinics of our University Hospital with severe RS virus respiratory disease. Diagnosis of RS virus disease was established by isolation of RS virus from the oropharynx, or a fourfold rise in RS virus serum antibodies during convalescence, or both as determined by one or more tests.

RESULTS

Quantitation of RS virus by IPSCA. Ten replicate microplate cultures of HELF cells were inoculated with $\log_{10}$ dilutions of cell-free stock virus in five different experiments. Two cultures inoculated with each virus dilution were fixed at the following times: 16, 24, 32, 40, 48 h after infection. Cell monolayers were then stained by the IPA technique. The number of single-stained

cells did not change between 16 and 24 h after infection, but the intensity of staining increased. After 24 h, the number of infectious foci increased, reaching at 48 h, about twice the number seen at 24 h and consisting of both single infected cells and small plaques of infected cells (in about the same proportion). At 24 h, stained infected cells were easily detected (Fig. 1) and their number was directly proportional to the virus inoculum (Fig. 2). No difference in infected cell counts was observed in the presence or absence of complement. The staining was mainly localized in the cytoplasm.

Specificity and reproducibility of neutralization test by IPSCA. The specificity of the neutralization test by IPSCA was verified by testing a RS virus preimmune and hyperimmune horse antiserum, acute- and convalescent-phase sera from three cases of RS virus infection in infants 6 to 12 months old and acute- and convalescent-phase sera from infants with acute respiratory infections due to influenza A (1 case) or B (two cases) virus, parainfluenza 1 (one case), 2 (one case) or 3 (three cases) virus and adenovirus (six cases). With RS virus-specific horse immune serum and sera from RS virus infections seroconversion was consistently observed, whereas in sera from patients with respiratory infections due to other viruses, neutralizing antibody titers against RS virus remained constant between acute- and convalescent-phase sera. The same results were obtained when test was performed in the presence of complement. The reproducibility of the test was determined by testing the same sera three times over a period of 3 months. Titers were consistently reproduced within a twofold dilution. Presence of complement did not influence reproducibility of the test.

Specificity and reproducibility of the IPA test for RS virus-specific IgG determination. Staining of RS virus-infected HEp-2 cell cultures in the IPA test for RS virus IgG showed a cytoplasmic pattern similar to that of infected HELF cell cultures (Fig. 1). The specificity of

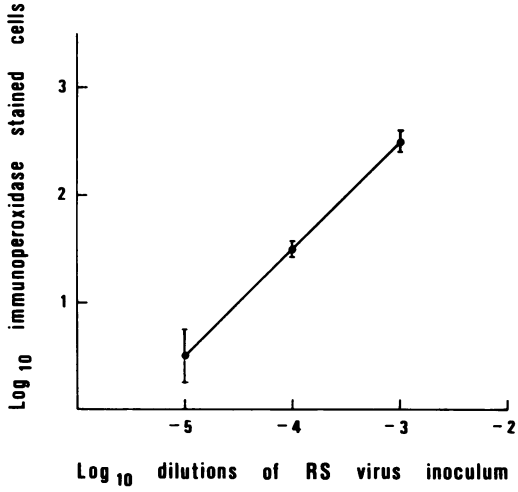

FIG. 2. *Dose-response curve for RS virus (Long strain) by the IPSCA. Points are arithmetic means (±standard deviation) of three experiments (10 replicates for each virus dilution in each experiment).*

FIG. 1. *RS virus-infected HELF cell culture: morphological patterns of cytoplasmic staining by the IPA technique at 24 h postinfection. ×250.*

the test was demonstrated by (i) absence of staining with preimmune horse serum and with acute-phase sera from children 6 to 12 months old suffering from RS virus acute respiratory disease; (ii) presence of staining up to high serum dilutions both with RS virus hyperimmune horse serum and with convalescent sera from the same children; (iii) similar titers of RS virus antibody in acute- and convalescent-phase sera from children with respiratory diseases produced by other viruses; (iv) blocking by hyperimmune horse serum of the reactivity of RS virus antigenic determinants with convalescent-phase sera from children with RS virus respiratory disease. This blocking test was done by incubating RS virus-infected cells first with horse serum, then with a high-titered RS virus convalescent serum, and finally with anti-human IgG conjugate. The virus staining reactivity of fixed microplate cell cultures stored at −80°C did not change over a period of 6 months. The reproducibility of the IPA test for RS virus IgG determination was confirmed by equivalent results (within a twofold dilution) obtained on paired sera tested at 1-month intervals for 3 months.

Comparison of different tests. Acute and convalescent-phase sera from 24 infants and young children hospitalized with RS virus respiratory disease were assayed by CF, IPA-IgG, and neutralization tests (in the presence and absence of complement) to study the relative efficiency of the four methods (Table 1). The IPA-IgG test was more sensitive than CF for detecting RS virus antibody, since 7 of 20 acute-phase sera with a CF antibody titer <1:8 had RS antibody at a titer of 1:16 or greater in the IPA-IgG test (Fig. 3A). In no instance was CF antibody detected in the absence of IPA-IgG antibody. Similarly, the neutralization test with IPSCA in the presence of complement detected RS virus antibody in 9 of 20 acute-phase sera with a CF titer <1:8, whereas the CF test did not detect antibody in any sera with a neutralizing titer <1:10 (Fig. 3B). Both tests detected RS virus antibody in three convalescent-phase sera, which were negative by CF.

Good agreement was generally observed between the IPA-IgG and the neutralization test in the presence of complement for detection of RS virus antibodies, although the latter usually gave higher titers (Fig. 3C). Two of the acute-phase sera which lacked detectable IPA-IgG antibody had antibody demonstrable by neutralization with complement. No serum with a neutralizing titer of <1:10 showed RS virus antibody by IPA-IgG. Similarly, a general agreement was found between neutralizing antibody titers in the presence and absence of complement, although 4 to 16-fold-higher titers were usually found in the presence of complement (Fig. 3D). In only one acute-phase serum was neutralizing antibody detected in the presence, but not in the absence, of complement. Comparison of RS virus neutralizing antibody titers in the presence and absence of complement in all 96 sera from 24 cases of acute respiratory disease showed that in the presence of complement titers were 4 to 16 times higher in approximately 80% of positive sera in the <1-, 5- to 12-, and 13- to 36-month age groups. In the 2- to 4-month group, more than 50% of the sera had titers within a twofold dilution in the two tests (Table 2).

Efficiency of neutralization by IPSCA and IPA-IgG tests for serodiagnosis of RS virus infections. The efficiency of CF, neutralization in the presence and absence of complement, and IPA-IgG for serodiagnosis of RS virus infection was compared (Table 3). In the <1-month-age group, RS virus was isolated from the oropharynx of each of the four patients, but no test demonstrated a significant serum antibody rise to RS virus. In the 2- to 4-month age group, only one of seven infants showed a significant rise in CF antibody. In contrast, the neutralization test in the absence of complement showed a significant antibody rise in five cases, and in the presence of complement in six cases. The IPA-IgG test detected a fourfold or greater antibody rise in all seven infants. The efficiency of the IPA-IgG test was also demonstrated in the two older age groups, where antibody rises were detected in all cases both by the IPA-IgG and the neutralization test with complement.

RS virus antibody response by different tests. Geometric mean titers (GMT) by different tests were compared during the course of RS virus respiratory disease (Fig. 4). In the <1-month age group, no significant variation in the GMT was observed by any test. In the 2- to 4-month age group, no fourfold rise in GMT was found by CF, but a rise greater than fourfold was observed with the IPA-IgG and neutralization tests in the presence and absence of complement between GMT of acute-phase sera and sera drawn at 11 to 20 days after onset of clinical symptoms. A fourfold or greater rise in GMT between acute- and convalescent-phase sera was detected by all four tests in two older age groups. Two of four cases in the 13- to 36-month age group were due to RS virus reinfection, as shown by presence of antibody by all tests in the acute-phase serum (Table 1). This is probably the reason for the higher GMT and higher GMT variability in all tests in the oldest age group as compared with younger age groups. With all

TABLE 1. *Antibody response to RS virus in 24 cases of RS virus acute respiratory disease, as determined by CF, IPA for IgG determination, and neutralization test with and without complement*

Age group (mo)	Case no. (age)	Respiratory disease	RS virus isolation	Sequential sera[a]	RS virus antibody titer by			
					CF	IPA-IgG	Nt[b]	Nt + complement
1	1,400 (2 weeks)	Bronchiolitis	+	I	8	256	80	160
				II	8	256	80	160
				III	8	256	80	320
				IV	8	256	80	160
	1,446 (2 weeks)	Bronchiolitis	+	I	16	256	320	1,280
				II	16	256	320	1,280
				III	16	128	320	1,280
				IV	16	256	320	1,280
	1,447 (2 weeks)	Bronchiolitis	+	I	<8	128	80	640
				II	<8	128	40	640
				III	8	128	80	640
				IV	8	128	80	640
	1,436 (4 weeks)	Bronchitis, otitis	+	I	<8	128	80	640
				II	<8	128	80	640
				III	<8	256	80	320
				IV	<8	256	80	640
2–4	1,392 (4 mo)	Bronchiolitis	+	I	<8	32	<10	20
				II	<8	128	10	80
				III	<8	512	80	160
				IV	<8	512	80	320
	1,466 (3 mo)	Bronchitis, otitis	+	I	<8	16	20	40
				II	<8	128	80	640
				III	8	256	80	1,280
				IV	8	256	80	1,280
	1,467 (4 mo)	Bronchitis	+	I	<8	<16	20	40
				II	<8	<16	20	40
				III	<8	128	80	80
				IV	8	128	80	320
	1,290 (3 mo)	Pneumonia	−	I	<8	32	20	80
				II	<8	64	40	160
				III	<8	128	320	640
				IV	<8	128	320	640
	1,334 (4 mo)	Bronchiolitis	−	I	<8	32	20	20
				II	<8	64	80	80
				III	8	128	320	320
				IV	8	128	320	640
	1,358 (2 mo)	Bronchiolitis	−	I	<8	<16	40	160
				II	<8	128	80	640
				III	16	512	80	640
				IV	32	512	80	640
	1,528 (3 mo)	Pneumonia, otitis	+	I	<8	32	80	160
				II	<8	128	80	160
				III	8	128	160	320
				IV	8	128	160	320
5–12	1,359 (10 mo)	Bronchiolitis	−	I	<8	<16	<10	<10
				II	<8	<16	<10	<10
				III	64	512	40	640
				IV	64	1024	640	1,280
	1,388 (12 mo)	Bronchiolitis	−	I	<8	<16	<10	<10
				II	<8	<16	<10	<10
				III	8	256	10	40
				IV	16	256	40	160
	1,404 (7 mo)	Bronchitis	+	I	<8	<16	<10	<10
				II	16	64	<10	20
				III	32	512	40	80
				IV	64	1,024	80	320

TABLE 1—*Continued*

Age group (mo)	Case no. (age)	Respiratory disease	RS virus isolation	Sequential sera[a]	RS virus antibody titer by			
					CF	IPA-IgG	Nt[b]	Nt + complement
	1,423 (7 mo)	Pneumonia	+	I	<8	<16	<10	<10
				II	8	256	20	80
				III	16	512	40	160
				IV	32	1,024	80	320
	1,431 (5 mo)	Bronchitis, otitis	+	I	<8	<16	<10	<10
				II	<8	<16	<10	<10
				III	16	128	10	80
				IV	16	512	40	320
	1,468 (9 mo)	Bronchitis	−	I	<8	<16	<10	<10
				II	<8	<16	<10	<10
				III	16	256	320	640
				IV	32	512	640	1,280
	1,474 (7 mo)	Bronchiolitis	−	I	<8	<16	<10	<10
				II	<8	128	<10	40
				III	512	256	20	320
				IV	512	256	40	320
	1,489 (9 mo)	Upper respir. tract infection, otitis	−	I	<8	<16	<10	<10
				II	<8	64	40	160
				III	<8	256	160	1,280
				IV	16	256	320	640
	1,490 (9 mo)	Upper respir. tract infection, otitis	−	I	<8	<16	<10	<10
				II	<8	64	40	160
				III	8	256	160	1,280
				IV	16	256	320	1,280
13–36	1,322 (36 mo)	Bronchitis	−	I	<8	<16	<10	<10
				II	8	128	10	40
				III	32	512	20	640
				IV	32	1,024	640	2,560
	1,349 (36 mo)	Bronchitis	−	I	16	64	80	320
				II	16	64	80	320
				III	256	4,096	5,120	20,480
				IV	256	4,096	5,120	20,480
	1,354 (20 mo)	Bronchiolitis	−	I	32	64	160	320
				II	128	512	640	1,280
				III	512	8,192	5,120	20,480
				IV	512	4,096	5,120	10,240
	1,441 (13 mo)	Bronchitis	+	I	<8	<16	<10	<10
				II	<8	256	40	320
				III	<8	256	40	320
				IV	8	512	40	320

[a] Sequential sera were drawn: I, within 4 days; II, at 5 to 10 days; III, at 11 to 20 days; and IV, at 21 to 40 days after onset of clinical symptoms.

[b] Nt, Neutralization test.

tests, peak titers were reached 20 days or later after onset of infection.

DISCUSSION

The present report describes two new techniques for determination of RS virus-specific antibodies: a rapid microneutralization test, which is completed in 24 h and is based on the count of RS virus-infected cells stained by the IPA technique, and the IPA technique for determination of RS virus IgG antibody, which is performed in 2 h by RS virus-infected ethanol-fixed microplate tissue cultures. Both tests appear to be specific, sensitive, and reproducible methods for detection of RS virus serum antibodies.

The neutralization test with IPSCA has the advantage over previous plaque reduction neutralization methods of giving earlier results. Because of the development of secondary foci between 24 and 48 h (13), the staining reaction must be performed 24 h after inoculation of cell cultures. The sensitivity of the test was improved in the presence of complement, since a

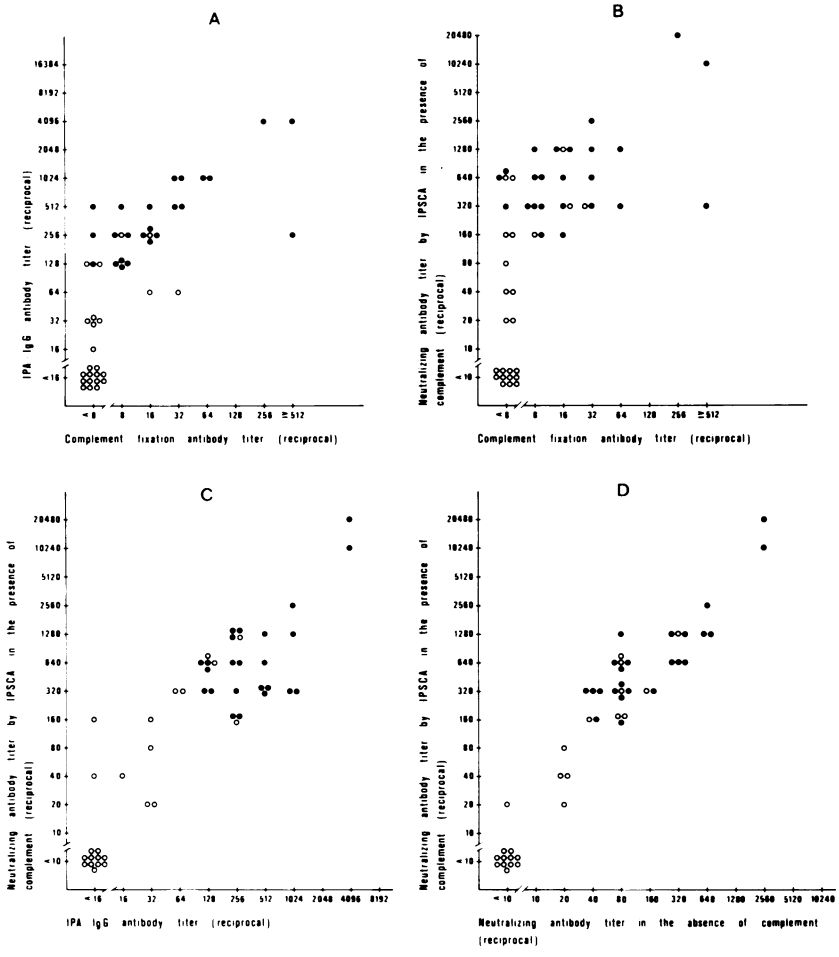

FIG. 3. *Comparison of RS virus antibodies determined by CF, IPA for IgG determination, and neutralization tests (Nt) by the IPSCA in the presence and absence of complement. Acute-* (○) *and convalescent-phase* (●) *sera were drawn from 24 infants and young children with acute RS virus respiratory disease.*

TABLE 2. *Comparison of RS virus neutralizing antibody titers in the presence and absence of complement in 96 sera from 24 cases of acute RS virus respiratory disease*

Age group (mo)	No. of sera (%) with antibody titer (reciprocal) ratio[a]:					Total
	1	2	4	8	16	
<1	0	3 (18.7)	6 (37.5)	6 (37.5)	1 (6.2)	16
2-4	4 (14.2)	11 (39.3)	6 (21.4)	5 (17.8)	2 (7.1)	28
5-12	13[b] (36.1)	5 (13.9)	10 (27.8)	7 (19.4)	1 (2.8)	36
13-36	3[c] (18.7)	2 (12.5)	7 (43.7)	4 (25.0)	0	16
Total	20 (28.8)	21 (21.9)	29 (30.2)	22 (22.9)	13 (13.5)	96

[a] Ratio, neutralization test with complement/neutralization test without complement.
[b] All 13 sera negative for presence of RS virus antibody by both tests.
[c] Two sera negative for presence of RS virus antibody by both tests. Titers of <8, <16, and <10 were considered to be titers of 4, 8, and 5, respectively.

slightly higher number of seroconversions was detected in infants, and most sera tested show higher titers. The complement-dependence of the RS virus neutralization reaction was much less evident in the 2- to 4-month age group of infants with acute RS virus respiratory infection than in infants infected during the first month of life and in children older than 5 months. The

reason for this finding remains obscure.

For the IPA-IgG test, HELF cell cultures were the most suitable cell system, since problems of nonspecific staining were minimal. The presence in two acute-phase sera of neutralizing antibody, which was not detected by the IPA-IgG test, is likely to have been due to RS virus immunoglobulin of the IgM class. In the 2- to 4-month age group, all seven cases of acute RS virus respiratory disease were detected by the IPA-IgG test, whereas one case was missed by neutralization with complement, and two cases were missed by neutralization without complement. A greater efficiency of the indirect immunofluorescence antibody technique for detecting specific RS virus antibody rises, as compared with CF and neutralization tests, has been reported (14). Recently, an enzyme-linked immunosorbent assay test for measurement of serological response to RS virus infections was shown to be more efficient than the plaque reduction and CF tests for detecting a serological response in young infants (1 to 3 months old) with severe RS virus respiratory disease (10). How the pres-

TABLE 3. *Serological response of patients with RS virus infection as measured by CF, neutralization with IPSCA in the presence and absence of complement, and the IPA-IgG technique*

Age group (mo)	No. tested	No. with rise in antibody titer by:			
		CF	Nt + complement[a]	Nt − complement	IPA-IgG
<1	4	0	0	0	0
2-4	7	1	6	5	7
5-12	9	8	9	8	9
13-36	4	3	4	4	4

[a] Nt, Neutralization test.

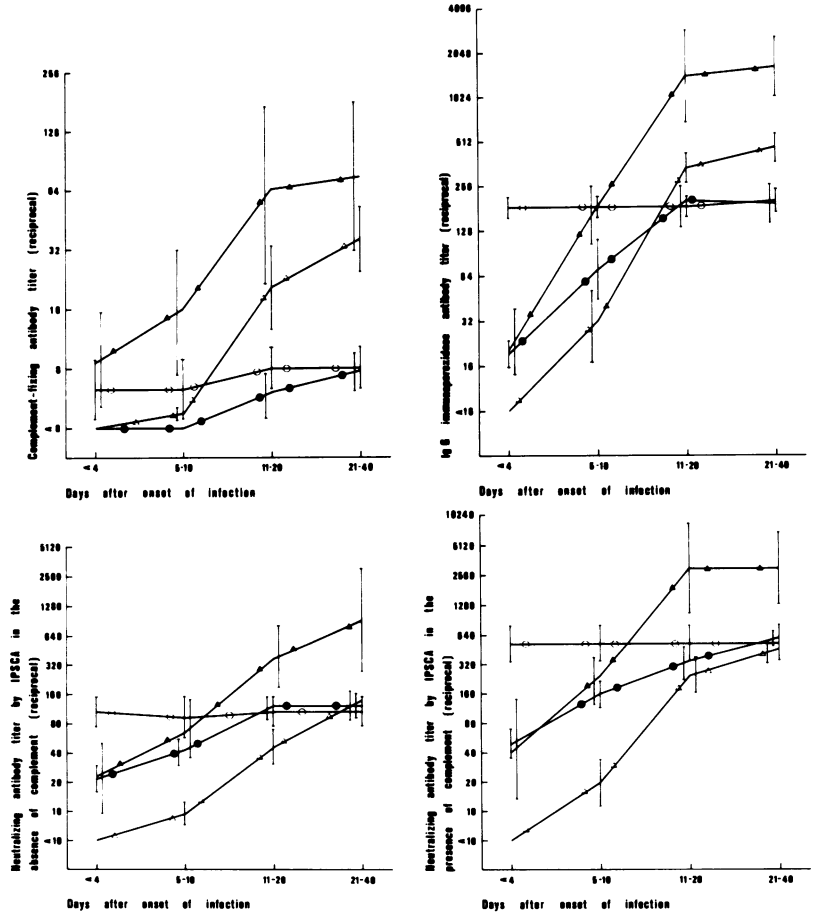

FIG. 4. *Geometric mean titers (±standard error of the mean) of RS virus CF, IPA-IgG, and neutralizing antibodies in the presence and absence of complement at indicated intervals from onset of acute respiratory disease in 24 infants and young children grouped by age (○, <1 month; ●, 2 to 4 months; △, 5 to 12 months; ▲, 13 to 36 months). Titers of <8, <16, and <10 were considered to be titers of 4, 8, and 5, respectively.*

ent results and those of ELISA tests are likely to compare remains to be determined.

The analysis of curves of RS virus GMT shows that in infants <1 month old no variation in the RS virus antibody titer was observed between the acute- and convalescent-phase sera by any test. The lack of expected fall in RS virus antibody titer in these infants would suggest a recent infection. After the first month of life, the majority of young infants appear capable of mounting an immune response to RS virus. In fact, in the 2- to 4-month age group, the curve of RS virus GMT showed an antibody titer rise greater than fourfold with all tests, except for CF.

We can conclude that the IPA-IgG test and the neutralization tests described here are essentially equivalent in their capability for detecting a significant rise in RS virus antibody in all cases tested of RS virus infection in infants older than 1 month. The IPA-IgG test appears to be the most rapid and the simplest test for serodiagnosis of RS virus infections in young infants. In addition, the IPA technique can be usefully employed for rapid RS virus identification in cell cultures.

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LITERATURE CITED

1. **Buynak, E. B., R. E. Weibel, A. J. Carlson, A. A. McLean, and M. R. Hilleman.** 1979. Further investigations of live respiratory syncytial virus vaccine administered parenterally. Proc. Soc. Exp. Biol. Med. **160:** 272-277.
2. **Center for Disease Control.** 1974. A guide to the performance of the standardized diagnostic complement fixation method and adaption to microtest, p. 1-56. Center for Disease Control, Atlanta.
3. **Chanock, R. M., H. W. Kim, A. J. Vargosko, A. Deleva, K. M. Johnson, C. Cumming, and R. H. Parrott.** 1961. Respiratory synctial virus. I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and minor respiratory diseases in children. J. Am. Med. Assoc. **176:**647-653.
4. **Gerna, G., G. Achilli, and R. W. Chambers.** 1977. Determination of neutralizing antibody and IgG antibody to varicella-zoster virus and IgG antibody to membrane antigens by the immunoperoxidase technique. J. Infect. Dis. **135:**975-979.
5. **Gerna, G., C. J. McCloud, and R. W. Chambers.** 1976. The immunoperoxidase technique for detection of antibodies to human cytomegalovirus. J. Clin. Microbiol. **3:**364-372.
6. **Kim, H. W., J. O. Arrobio, G. Pyles, C. D. Brandt, E. Camargo, R. M. Chanock, and R. H. Parrott.** 1971. Clinical and immunological response of infants and children to administration of low-temperature adapted respiratory syncytial virus. Pediatrics **48:**745-755.
7. **Mills, J., J. E. van Kirk, P. F. Wright, and R. M. Chanock.** 1971. Experimental respiratory syncytial virus infection of adults. J. Immunol. **107:**123-130.
8. **Parrott, R. H., H. W. Kim, J. O. Arrobio, D. S. Hodes, B. R. Murphy, C. D. Brandt, E. Camargo, and R. M. Chanock.** 1973. Epidemiology of respiratory syncytial virus infections in Washington, D.C. II. Infection and disease with respect to age, immunologic status, race and sex. Am. J. Epidemiol. **98:**289-300.
9. **Parrott, R. H., A. J. Vargosko, H. W. Kim, C. Cumming, H. Turner, R. J. Huebner, and R. M. Chanock.** 1961. Respiratory syncytial virus. II. Serological studies over a 34-month period of children with bronchiolitis, pneumonia, and minor respiratory disease. J. Am. Med. Assoc. **176:**653-657.
10. **Richardson, L. S., R. H. Yolken, R. B. Belshe, E. Camargo, H. W. Kim, and R. M. Chanock.** 1978. Enzyme-linked immunosorbent assay for measurement of serological response to respiratory syncytial virus infections. Infect. Immun. **20:**660-664.
11. **Ross, C. A. C., I. W. Pinkerton, and F. A. Assaad.** 1971. Pathogenesis of respiratory syncytial virus disease in infancy. Arch. Dis. Child. **46:**702-704.
12. **Ross, C. A. C., E. J. Stott, S. McMichael, and I. A. Crowther.** 1964. Problems of laboratory diagnosis of respiratory syncytial virus infection in childhood. Arch. Gesamte Virusforsch. **14:**553-562.
13. **Schieble, J. H., E. H. Lennette, and A. Kase.** 1965. An immunofluorescent staining method for rapid identification of respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. **120:**203-208.
14. **Urquhart, G. E. D., and G. H. Walker.** 1972. Immunofluorescence for routine diagnosis of respiratory syncytial virus infection. J. Clin. Pathol. **25:**843-845.