Production of Antiserum to Respiratory Syncytial Virus Polypeptides: Application in Enzyme-Linked Immunosorbent Assay

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By use of crossed immunoelectrophoresis techniques, respiratory syncytial (RS) virus-specific precipitates were produced between RS virus cellular antigen [solubilized in tris(hydroxymethyl)aminomethane-glycine buffer, pH 9] and antiserum raised in rabbits against semipurified RS virus. When these precipitates were employed as antigens for further immunizations in rabbits, antibodies (anti-RS-precip.I) were produced which reacted with only one RS virus antigen when tested by the crossed immunoelectrophoresis technique. Precipitates obtained between RS virus cellular antigen (labeled with L[35S]methionine) and anti-RS-precip.I were examined by polyacrylamide gel electrophoresis, which showed that anti-RS-precip.I precipitated RS virus polypeptides of molecular weights 28,000 to 84,000. Anti-RS-precip.I was employed as capture antibodies in the enzyme-linked immunosorbent assay, in which RS virus cellular antigen was used as the second layer. Determination of human RS virus immunoglobulin G antibodies by this enzyme-linked immunosorbent assay technique showed a high degree of sensitivity, specificity, and reproducibility.

The introduction of new sensitive enzyme immunoassays (2, 17) into diagnostic virology has created a demand for more potent and more specific immune sera. The purpose of the present report is to describe the production of antiserum to respiratory syncytial (RS) virus polypeptides (molecular weight [MW], 28,000 to 84,000) by the use of crossed immunoelectrophoresis (CIEP) techniques. Further characterization of this antiserum and a brief summary of results obtained with it in the enzyme-linked immunosorbent assay (ELISA) will also be presented.

RS virus is a single-stranded ribonucleic acid virus belonging to the family Paramyxoviridae (14). Multiplication occurs in the cytoplasm of the host cell, independent of cellular deoxyribonucleic acid (11), with maturation taking place by budding from altered plasma membranes (16). Previous reports have shown that six to eight RS virus polypeptides (MW ranging from 20,000 to 80,000) can be demonstrated in RS virions or in RS virus-infected cells by polyacrylamide gel electrophoresis (PAGE) (4, 5, 13, 18, 24, 32). According to a recent World Health Organization report, RS virus is the most important respiratory disease pathogen of infants and young children (31).

It is difficult to produce RS virions or RS viral antigens free of host cell material. CIEP techniques (30) facilitate the purification of viral antigens, as shown by previous reports from this laboratory (8, 9, 12, 25). The immunization of rabbits with precipitates (consisting of rabbit antisera and viral antigens) obtained by CIEP techniques has made it possible to produce specific antisera to several herpes simplex virus (HSV) antigens (27) as well as to rotavirus (8) and influenza virus antigens (12).

MATERIALS AND METHODS

Cell culture. Vero and HEp-2 cells were grown in Roux bottles with Eagle minimal essential medium (Earles salt solution) and 10% fetal bovine serum and maintained in the same medium with 2% fetal bovine serum. For isolation of RS virus from specimens of nasopharyngeal secretions from patients with acute respiratory disease, HEp-2 cells were grown in plastic tubes (12 by 100 mm; Nunc, Denmark).

RS virus strain. The Long strain of RS virus, passed approximately 5 times in KB cells and approximately 55 times in HEp-2 cells, was used in these studies.

Titration of RS virus. Virus titration was done by plaque titration in Vero cell monolayers in 2.5-cm petri
dishes. RS virus was passed twice in Vero cell cultures (Roux bottles), and tissue culture maintenance medium was harvested from cultures showing 3+ cytotoxic effect. The maintenance medium was clarified by low-speed centrifugation and supplemented with bovine serum albumin (final concentration, 0.1%) before being stored in small volumes at −70°C. Virus diluted in tissue culture maintenance medium was absorbed for 2 h (at 35°C in a CO₂ incubator). After removal of the rest of the inoculum from the petri dishes, Leibovitz L-15 medium containing 10% fetal bovine serum and 0.8% agarose (19) was overlaid. After 5 days of incubation at 35°C without CO₂, the monolayers were fixed with 100% methyl alcohol for 10 min, stained with methylene blue (1%, wt/vol, in distilled water) for 30 min after removal of the overlay, and destained for 5 min with 96% ethyl alcohol.

Titration of RS virus neutralizing antibodies. Antibody titration was done by plaque reduction tests. Vero cell culture maintenance medium containing approximately 100 plaque-forming units per 0.3 ml was incubated with equal volumes of the serum dilutions at room temperature. After 1 h, 0.6 ml of these mixtures was added to each Vero cell monolayer (in 2.5-cm petri dishes) and incubated for 2 h in a CO₂ incubator at 37°C before the rest of the inoculum was removed and the Leibovitz-agarose overlay described above was added. The titer was read after 5 days of incubation at 35°C without CO₂ and calculated as the highest serum dilution giving more than 50% reduction of the plaque-forming unit count when compared with control dishes without serum.

Production of antiserum to gradient-purified RS virus. One-liter volumes of tissue culture maintenance medium from RS virus-infected HEp-2 cell monolayers in Roux bottles (showing 3+ cytotoxic effect) were treated according to the method described by Senterfit and Baldrige (22). After precipitation with polyethylene glycol (PEG 6,000), the resuspended sediment was ultracentrifuged (26,000 rpm) for 16 h at 4°C (Beckman SW 27) on a continuous 30 to 70% (wt/vol) sucrose gradient made in tris(hydroxymethyl)aminomethane (Tris)-ethylenediaminetetraacetate buffer as described (22). Virus-containing bands (1.20 to 1.22 g/ml) were harvested, diluted with phosphate-buffered saline (PBS), and sedimented by further ultracentrifugation at 25,000 rpm for 3 h (Beckman SW 27) at 4°C. This final sediment, diluted in a small volume of PBS, contained approximately 300 × 10⁶ virions per ml (checked by electron microscopy; see below) and constituted the antigen.

Rabbits were given four intracutaneous injections (at intervals of 10 days) of approximately 30 × 10⁶ virions in PBS, which had been mixed with equal volumes of complete Freund adjuvant and sonicated for 30 s (Branson B12 sonifier, 18 kilocycles per s) just before the immunizations. Serum harvested 8 days after the last immunization is referred to as anti-RSV-prim.

Electron microscopy. Estimation of the number of RS virions was made according to the method described by Pyrhönen (20). Volumes (10 μl) of serial virus dilutions were layered onto 200-square grids, and virus was allowed to absorb for 30 s. Excess fluid was drained by filter paper, and the grids were flooded twice with contrast (2% solution of ammonium molybdate in water). After drying, the grids were examined in a Philips Transmission EM 301 at a primary magnification of 45,000. At least 10 squares were examined. Thus a titer could be read as the highest dilution still containing intact virus particles.

RS virus cellular antigen employed in CIEP techniques and in ELISA. RS virus HEp-2 cell monolayers showing 3+ cytotoxic effect were gently washed twice in PBS and harvested in Tris-glycine buffer (0.08 M Tris, 0.02 M glycine; pH 9), 1 ml per Roux bottle. The harvested cells were frozen and thawed three times and sonicated twice (30 s each time at 18 kilocycles per s on ice in a Branson B12 sonifier). After further centrifugation at 5000 × g for 30 min (Sorvall S-3 Automatic) at 4°C, the supernatant fluid constituted the antigen. Control antigen was made in exactly the same way from uninfected HEp-2 cells.

CIEP. The CIEP technique was performed as described previously (8, 25, 30). Details regarding the exact experimental conditions are given in the figure legends.

Immunization of rabbits with agarose gel precipitate produced by CIEP. RSV precipitate I (shown in Fig. 1A) was used as the antigen. Each rabbit was immunized intracutaneously three times (with three precipitates each time) at intervals of 10 days and bled 10 days after the last immunization, as described earlier (12, 26).

Human serum samples examined. Paired serum samples examined were from 7 children (1 to 6 years old) admitted to the pediatric department of Copenhagen County Hospital (Gentofte) for acute lower respiratory disease. The first serum sample was taken on admission, and the second was taken approximately 4 weeks later.

Titration of RS virus antibodies by indirect immunofluorescence (IF). RS virus-infected HEp-2 cell monolayers on cover slips in Layton tubes, infected 2 days earlier at a multiplicity of infection of 0.1, were washed twice with PBS and fixed in acetone for 10 min at room temperature. Dilutions of human sera were added for 1 h at 37°C. An optimal dilution of fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin G (IgG) (DAKO Immunoglobulins A/S, Copenhagen; code no. F1090) was added for 45 min at 37°C. The titer was defined as the highest dilution of patient’s serum giving definite bright fluorescence of granular inclusions in the cytoplasm.

Labeling of RS virus-infected HEp-2 cells with L-[³⁵S]methionine. One Roux bottle infected 48 h earlier at a multiplicity of infection of 0.05 was incubated (at 35°C) with 25 ml of maintenance medium containing 25% of the normal concentration of methionine, L-[³⁵S]methionine (0.5 mCi/ml; New England Nuclear Corp., lot no. 1271-172; specific activity, 1,010.6 Ci/mmol, 1.0 mCi in 0.1 ml) was added to this bottle, and after further incubation for 24 h, the cells and medium were harvested. The cells showed advanced cytopathic effect and were beginning to detach from the bottle when they were harvested. The labeled HEp-2 cells were mixed with RS virus-infected HEp-
2 cells from another Roux bottle cultivated simultaneously, but without isotope and with normal methionine concentration in the maintenance medium (25 ml). RS virus cellular antigen was produced from this mixture of labeled and unlabeled cells as described above. The media from the labeled and unlabeled bottles were mixed, and RS virus was purified from this mixture as described below.

**Purification of L-[35S]methionine-labeled RS virus.** The mixture of tissue culture maintenance media referred to above was clarified (3,000 × g, 30 min, 4°C), and 25 ml was centrifuged for 90 min at 26,000 × g (SW28.1 rotor, Beckman ultracentrifuge L-2-65 B) at 4°C on a double cushion of sucrose. This consisted of 5 ml of 65% (wt/vol) sucrose and 5 ml of 20% (wt/vol) sucrose made up in 0.01 M HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid), 0.5 M NaCl, and 0.01 M ethylenediaminetetraacetate (pH 7.4). The virus-containing band was isolated, diluted to 10 ml with 0.01 M HEPES and 0.002 M ethylenediaminetetraacetate (pH 7.4), layered on a 20 to 50% (wt/vol) continuous potassium tartrate gradient, and centrifuged for 18 h (4°C) at 22,000 × g in the rotor indicated above. This gradient was made up in 0.01 M HEPES and 0.02 M ethylenediaminetetraacetate (pH 7.4). Twenty-eight fractions were harvested from the gradient. A 200-μl sample of the peak fraction (showing maximum counts per minute and a specific gravity of 1.21 g/ml) was diluted up to 4 ml in PBS containing 1% of the protein inhibitor aprotinin (Midran, Novo Industries, Copenhagen, Denmark) and centrifuged for 1 h at 55,000 × g (SW60 rotor) at 4°C. The sediment was suspended in 50 μl of electrophoresis sample buffer (12) containing 2% aprotinin before being tested by analytical sodium dodecyl sulfate (SDS)-PAGE as described below.

**SDS-PAGE.** Analytical SDS-PAGE of solubilized samples of purified RS virus and of solubilized gel precipitates was carried out in vertical slab gels as described previously (12), with the following modifications: the thickness of the gel was 1.5 mm, the stacking gel contained 4% (wt/vol) acrylamide, and the separation gel contained 10% (wt/vol) acrylamide.

The sample buffer was supplemented with 2% (wt/vol) aprotinin; the electrophoresis was carried out for 260 min with a constant current of 40 mA. Autoradiography was carried out at −70°C for 2 weeks with Kodak XRY-1 film.

The preparation of the immunoglobulin fraction from rabbit antisera and from human sera. Immunoglobulin fractions were obtained by the method described by Harboe and Inglis (10).

**Determination of concentrations of immunoglobulin solutions.** Immunoglobulin concentrations were determined by the method described by Bramhall et al. (3).

**Examination of nasopharyngeal secretions from children for cells containing RS virus antigen.** Nasopharyngeal secretions were examined by the method described by Gardner and McQuillan (6). Washed cells fixed on ordinary microscope slides were incubated with anti-RS serum (bovine serum; Wellcome Research Laboratories, Beckenham, England) at 37°C for 1 h, followed by incubation (also at 37°C) for 45 min with fluorescein isothiocyanate-conjugated rabbit anti-bovine serum (Wellcome).

**ELISA employed.** Determination of IgG antibodies in human sera was performed on polystyrene microtest plates (A/S Nunc, Denmark; code no. 2-62162) by employing the following reagents: (i) rabbit anti-RS virus precipitate I serum (anti-RSV-precip.I; see below), immunoglobulin fraction containing 35 mg of immunoglobulin per ml; (ii) RS virus cellular antigen (or control antigen) harvested in Tris-glycine buffer (pH 9) as described above; (iii) rabbit anti-human IgG, peroxidase conjugated (DAKO immunoglobulins A/S; code no. P1090); (iv) bicarbonate buffer (pH 9.6) (15 mM Na2CO3, 35 mM NaHCO3, 3 mM Na3), (v) washing solution (pH 7.2) (0.5 M NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 6.5 mM Na2HPO4, 2H2O, and 0.5 ml of Tween-20 added per 1,000 ml); (vi) dilution buffer (pH 7.2) (same as washing solution, with 5 g of bovine albumin [Sigma A8022, fraction V] and 10 ml of phenol red [0.1%, wt/vol] added per 1,000 ml); (vii) staining solution (pH 5) (34.7 mM citric acid, 66.7 mM Na2HPO4, 2H2O, 500 mg of 1,2-phenylenediamine-dihydrochloride [Fluka], and 500 μl of 30% H2O2 added per 1,000 ml).

Serum dilutions of 1:100, 1:400, 1:1,600, and 1:6,400 were examined by the following procedure utilizing the reagents described above. (i) The wells were coated with 1 μl of each rabbit anti-RS virus serum diluted 1:5,000 in bicarbonate buffer. (ii) The plate was washed four times for 1 min (with intervals of 1 min) in washing solution. Between fillings the plate was tapped, upside down, against soft tissue. (iii) RSV antigen or control antigen diluted 1:100 in dilution buffer was added to the wells (100 μl per well) overnight at 4°C. (iv) The plate was washed four times again (see step ii). (v) Human serum samples diluted in washing solution were added to the plate for 1 h at room temperature. (vi) The washing procedure was repeated. (vii) Anti-human IgG (reagent 3), diluted 1:1,000 in dilution buffer, was added for 1 h at room temperature (100 μl per well). (viii) The washing procedure was repeated. (ix) Staining solution without H2O2 and phenylenediamine was added to the plate for 1 to 2 min. (x) Complete staining solution was added to the plate (100 μl per well) for 4 min. (xi) The reaction was stopped by adding 150 μl of 2 M H2SO4 per well. The results were read in a Vitatron Universal photometer at 472 nm, but positive or negative results and increase or no increase of optical density (OD) values between serum samples could usually be read by the naked eye. Definite increase of IgG antibody concentrations between two serum samples as determined by the ELISA described represents more than a doubling of the OD values. The same positive human standard serum was run on all plates. The small (approximately 5 to 10%) day-to-day variation of the OD values was corrected by comparing the day-to-day results obtained with this positive serum. All serum dilutions were tested with the control antigen also. The final OD values of the positive sera were obtained after subtraction of the values found with control antigen. More than 90% of the OD values obtained with serum dilutions of 1:400 and with control antigen were less than 0.1, and background values for the
plates (without antigen or serum added) ranged from 0.005 to 0.015.

Titration of CF RS virus antibodies. Complement-fixing (CF) antibody titration was done at the State Serum Institute in Copenhagen (by C. Mor

dholtar) according to a method described earlier (15). The antigen employed was made from RS virus (Long strain)-infected GMK cell cultures.

HSV type 1 reference antigen and antiserum against HSV antigen no. 11. Reference antigen was prepared from Triton X-100-solubilized rabbit cornea cells as described earlier (25, 27). Antiserum against the glycoprotein, antigen no. 11, was raised in rabbits as described earlier (27, 29).

RESULTS

Production of immunoprecipitates by CIEP between antibodies to semipurified RS virus (anti-RSV-prim) and RS virus antigen. Figure 1 (A and B) shows the results of CIEP in which anti-RSV-prim was employed in both plates in the second-dimension gel. RS virus antigen was employed in the first-dimension gel in Fig. 1A, and control antigen was employed in Fig. 1B.

The antibodies used in these plates were adsorbed with HEp-2 cells before use (6 × 10⁷ cells per ml of serum for 18 h at 4°C). The antigens used were cellular antigens sonicated in Tris-glycine buffer as outlined above. In this figure and in Fig. 2 an empty gel (without antigen or serum) was interposed between the first- and second-dimension gels to improve the precipi-
tate patterns.

The precipitate marked RSV-precip. I, which could be found only in the RS antigen plate, was used for further immunization. This precipitate was cut out of the wet gel, and several immuniza-
tions with precipitates were given intracuta-
neously to rabbits as described above. The antiserum obtained by this procedure is called anti-RSV-precip. I.

Characterization of rabbit anti-RSV-precip. I. (i) Lack of reaction with RS virus glycoproteins in CIEP. Figure 2 shows the precipitate obtained by CIEP, with the same antigen as in Fig. 1 in the first-dimension gel and with anti-RSV-precip. I antibodies in the second-dimension gel; the immunoglobulin fraction was adjusted to a concentration of approximately 30 g/liter. Figure 2B is analogous to Fig. 2A, except that the lectin concanavalin A (ConA) was incorpo-
rated in the first-dimension gel. ConA was employed at a concentration known to absorb virus antigens of glycoprotein nature (12, 28). The similarity of the precipitates in Fig. 2A and B demonstrates that the antibodies employed (anti-RSV-precip. I) did not precipitate out any glycoproteins from the antigen when a concen-

![Fig. 1.](http://jcm.asm.org/)

**Fig. 1.** (A) CIEP of RS virus HEp-2 cellular antigen. First-dimension gel: 60 μl of the antigen preparation was electrophoresed in 1.5-mm-thick 1% (wt/vol) agarose gel as described above, containing rabbit antibodies (anti-RSV-prim) to RS virus. Anti-RSV-prim was used as the immunoglobulin fraction at 15 μl/cm² of gel, as a solution (in 0.9% NaCl) containing 20 g of immunoglobulin per liter. Current was 2 V/cm of gel for 18 h. Electrophoretic buffer was the same as the gel buffer. Staining was with Coomassie brilliant blue. This and the following CIEP experiments shown were all performed on 7-by-10-cm glass plates. An intermediate gel section (30 by 70 by 1.4 mm) was interposed between the first- and second-dimension gels. (B) This gel is analogous to that in (A), except that the control HEp-2 cellular antigen was electrophoresed in the first-dimension gel.

tration of ConA of 60 μg/cm² was employed in the first-dimension gel. When antiserum against the glycoprotein antigen, HSV antigen no. 11, was added to the second-dimension gel and HSV type 1 reference antigen was added to the first-dimension gel, antigen no. 11 was precipitated out (to the left in the intermediate gel in Fig. 2A). This precipitate was removed in Fig. 2B by the ConA and consequently constituted a posi-
(ii) The immunogenic potency in human RS virus infection of the precipitated antigen. The anti-RSV-precip.I immunoglobulins were produced for diagnostic purposes. It was therefore important to know whether antibodies to the antigen precipitated out by CIEP with rabbit antibodies (as in Fig. 2A) were also produced in human infections. This question was evaluated by the CIEP-intermediate gel technique (1).

In Fig. 3 the same antibodies and antigen were employed as in Fig. 2A. In Fig. 3A the intermediate gel is empty; in Fig. 3B and C the immunoglobulin fractions of human sera (15 μl/cm²) were incorporated into this gel section. In Fig. 3B the immunoglobulin fraction employed was prepared from a pool of 10 human serum sam-
samples without antibodies to RS virus (checked by CF, IF, and ELISA techniques). In Fig. 3C the immunoglobulin fraction added to the intermediate gel was prepared from a pool of 10 human serum samples with intermediate to high antibody titer to virus. The immunoglobulin concentrations of these pools were adjusted to approximately 10 g/liter. All 20 sera were from children (1 to 3 years old) with lower respiratory disease (pneumonitis or bronchitis). A comparison of Fig. 3A and Fig. 3C reveals that the antigen migration was inhibited in the intermediate gel in Fig. 3C (containing human antibodies to RS virus), whereas no inhibition was seen in Fig. 3B. These findings strongly indicate that the antigens reacting in precipitation with rabbit anti-RS-precip.I antibodies are also immunogenic during RS virus infections in children.

(iii) Antibody titers measured by plaque neutralization and IF technique. When the immunoglobulin fraction (30 g/liter) from anti-RS-precip.I serum was tested by plaque reduction in Vero cell monolayers, the titer was found to be less than 1:50. For comparison, the positive and negative human serum pools (described above) showed plaque reduction titers of approximately 1:3,200 and less than 1:50, respectively.

The titer of the immunoglobulin fraction of anti-RS-precip.I serum (30 g/liter) determined by the IF technique was 1:320, whereas the immunoglobulin fractions of the positive and negative human serum pools (10 g/liter) were 1:160 and less than 1:10, respectively. Anti-RS-precip.I antibodies gave a fine granular cytoplasmic fluorescence in the HEp-2 cells used in the IF assay.

(iv) Determination of MW of RS virus polypeptides precipitated by anti-RS-precip.I antibodies. Polypeptide MWs were determined by PAGE in vertical slab gels with L-$^{35}$S methionine-labeled RS virus and precipitates formed between L-$^{35}$S methionine-labeled RS virus cellular antigen and anti-RS-precip.I Part A and part B of the precipitate shown in Fig. 4 were cut out of the wet gel and solubilized in SDS-containing Tris buffer before being tested. Anti-RS-precip.I did not produce precipitates with cellular antigen produced from uninfected control HEp-2 cell cultures. Therefore precipitates produced with control antigen from L-$^{35}$S methionine-labeled control cultures could not be tested. The gels were calibrated with a mixture of six proteins (MW 14,400 to 94,000) run at the same time (12). Figure 5 shows autoradiography of solubilized RS virus (track c). The samples, which were layered on the gel corresponding to tracks a, b, and c, contained approximately 100,000, 40,000, and 40,000 cpm, respectively. Several of the polypeptides shown in track a were also found in tracks b and c. A polypeptide of MW 28,000 was seen in track c and in track a but was scarcely visible in track b.

\[ \text{FIG. 4. CIEP of L-$^{35}$S methionine-labeled RS virus cellular antigen. First-dimension gel: 75 \mu l of the antigenic preparation was electrophoresed. Second-dimension gel: anti-RS-precip.I employed in the same way as described for Fig. 2 and 3. Experimental conditions were otherwise as described for Fig. 1.} \]

\[ \text{FIG. 5. SDS-PAGE performed with L-$^{35}$S methionine-labeled RS virus and gel precipitates consisting of L-$^{35}$S methionine-labeled RS virus cellular antigen and anti-RS-precip.I. (a) solubilized purified RS virus; (b) solubilized precipitate, part A of precipitate shown in Fig. 4; (c) solubilized precipitate, part B of precipitate shown in Fig. 4.} \]
Determination of IgG antibodies in human RS virus infections by use of anti-RSV-precip.I as capture antibodies in ELISA. Serum pairs from 147 infants and children admitted to hospital for lower respiratory tract disease were tested for RS virus IgG antibodies by ELISA. The technique employed is described in detail above. The majority of the patients were less than 2 years old. In 46 of the patients the ELISA showed increases of RS antibody concentration. In Table 1 the results of various diagnostic tests performed on sera and samples of nasopharyngeal secretions from these 46 patients are summarized. In 26 of the patients RS virus could be found in nasopharyngeal secretions, either by direct immunofluorescent examination of epithelial cells (present in the secretions) or by inoculation of a small amount of secretion into each of two HEp-2 tube cultures. Thirty-two of the paired serum samples showed titer increases of RS virus IgG antibodies when examined by the indirect IF technique. In eight patients, only ELISA gave a definite diagnosis of RS virus infection. No patients with titer increases by other tests (CF or IF techniques) were negative by ELISA.

**DISCUSSION**

Vertical slab gel PAGE of precipitates produced between RS virus HEp-2 cellular antigen and rabbit anti-RSV-precip.I (raised against gel precipitates designated RSV-precip.I) showed that anti-RSV-precip.I reacted in the CIEP assay with six polypeptides which had MWs ranging from approximately 84,000. Wunner and Pringle (32) and Cash et al. (4, 5) have described seven RS virus polypeptides (MW 25,000 to 48,000) prepared from RS virus grown in BSC-1 cells. These authors claim that the three predominant polypeptides were a glycoprotein of MW 48,000 (VGP 48), a nucleocapside-polypeptide of MW 41,000 (VP 41), and a polypeptide of MW 27,000 (VP 27). Levine (13) examined RS virus produced in HeLa cells, found (by PAGE) seven RS virus polypeptides of MW 22,000 to 79,000, and described three of these polypeptides (MW 22,000, 56,000, 79,000) as glycoproteins. However, in a recent publication, Peeples and Levine (18) modified their PAGE technique and obtained MWs for the two large glycoproteins of 49,000 and 90,000. These authors furthermore reported that the dominant nucleocapsid polypeptide has a MW of 44,000 (18). Ueba (24) advocated that RS virus precipitated by PEG 6,000 should be filtered through a Bio-Rad Biogel A 15-m column before further purification on sucrose gradients. This author (24) found (by PAGE) seven RS virus polypeptides of MW 25,000 to 75,000.

In the present paper, Tris-glycine buffer without detergent was employed for the preparation of the antigen used in CIEP techniques. When ConA was added to the first-dimension gel in CIEP, no inhibiting effect was observed on the antigens precipitated by anti-RSV-precip.I but ConA did inhibit the precipitation of HSV antigen no. 11, which was included in the same plate as a positive control. This antigen is a glycoprotein, according to earlier findings (27). Furthermore, anti-RSV-precip.I did not neutralize RS virus when assayed in plaque-reduction tests. These findings do not support the possibility that anti-RSV-precip.I is able to react with RS virus surface glycoproteins.

Levine (13) and Peeples and Levine (18) examined RS virus polypeptides by different PAGE techniques and showed that the migration of the glycoproteins in the discontinuous buffer system of Laemmli was different from the migration of the glycoproteins observed when a neutral buffer system was employed. The buffer system of Laemmli was also employed in the

**TABLE 1. Forty-six serum pairs showing increase in OD when tested for RS virus IgG antibodies by ELISA, compared with other diagnostic tests**

<table>
<thead>
<tr>
<th>Results</th>
<th>No. of serum pairs showing</th>
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<tr>
<td></td>
<td>Titer increase by CF (24.9)*</td>
</tr>
<tr>
<td>OD increase by ELISA</td>
<td>28*</td>
</tr>
<tr>
<td>RS virus in secretion (by IF or isolation)</td>
<td>17</td>
</tr>
<tr>
<td>Titer increase by IF</td>
<td>28*</td>
</tr>
<tr>
<td>No RSV in secretion, no titer increase by IF</td>
<td>0</td>
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</table>

* Range of OD values for first sera (dilution 1:400 examined) in 46 serum pairs, 0.000 to 0.796; mean, 0.174. Range of OD values for second sera (dilution 1:400 examined) in 46 serum pairs, 0.261 to 1.968; mean, 0.848.
† Mean number of days between blood samples.
‡ <4 to 4.
§ Mean age of children in the group, 18.9 months.
¶ Mean age, 13.9 months.
** Mean age, 13.1 months.
present investigation, but with some modifications as described above. Tyrrell et al. (23) examined measles virus polypeptides by SDS-PAGE and found that the addition of the protease inhibitor aprotinin reduced the breakdown of some of the polypeptides during processing of the material. In the present report, aprotinin was added both to the gel where labeled RS virus precipitates were produced and to the sample buffer used for the preparation of the samples for SDS-PAGE. Protease inhibitors have not been employed in previously published investigations concerning RS virus polypeptides.

Differences in PAGE techniques employed (as mentioned above) and other differences in methods (e.g., different cell cultures and the use of actinomycin D in cell culture medium by some investigators) can probably explain the different MWs for RS virus polypeptides published by different groups (13, 18, 24, 32). The MW for RS virus polypeptides determined with gel precipitates, as reported in the present paper, also differs slightly from the MW previously published. MWs for these polypeptides (shown in Fig. 5, tracks b and c, in this report) fall into the same range as the MWs reported by Peeples and Levine (18) and by Ueba (24). The limited number of PAGE experiments presented in this article is not sufficient for any identification of the RS virus polypeptides found. These experiments nevertheless show that the antiprecipitate hyperimmune serum produced (anti-RSV-precip.I) precipitates only polypeptides from RS virus-infected HEp-2 cells which are also present in purified RS virus.

CIEP techniques using human IgG (with and without RS virus antibodies) added to intermediate gel sections have shown that the polypeptides, or at least some of them, precipitated by anti-RSV-precip.I are immunogenic during RS virus infections in children. This statement is supported by the great sensitivity and specificity of ELISA when these polypeptides are employed as antigens for measuring RS virus IgG antibodies in infants and small children.

Gerna et al. (7) measured RS virus IgG antibody by an immunoperoxidase technique in which fixed HEp-2 cells or human embryonic lung fibroblasts in tissue culture microplates were employed as antigen. They found that the immunoperoxidase technique was more sensitive than the CF technique for the detection of antibody rises, especially in 2- to 4-month-old patients.

Richardson et al. (21) measured RS antibodies by ELISA using a suspension of freeze-thawed RS virus-infected HEp-2 or GMK cells (in tissue culture maintenance medium) as antigen absorbed directly to the plate in carbonate buffer (pH 9.8). They diluted the serum samples in PBS-Tween, supplemented with 1% fetal bovine serum and 19% of an uninfected cell suspension, to reduce nonspecific binding of the test serum (21). They found that the ELISA technique was capable of detecting serological responses, especially in young infants 1 to 3 months old where these responses could be demonstrated only with difficulty by use of either CF or plaque-reduction techniques (21). These authors nevertheless could not demonstrate increased sensitivity of ELISA (as compared with the other techniques mentioned) in children more than 7 months old (21). In the present report, RS virus IgG antibodies have been determined by ELISA with more well-defined RSV virus antibodies and antigens employed as first and second layers on microtest plates. When this methodology was employed, ELISA also showed greater sensitivity than other diagnostic tests (serology by CF or IF techniques, nasopharyngeal secretions examined by direct immunofluorescence or by virus culture) in children more than 1 year old. Furthermore, this technique showed good reproducibility, low background OD values, and low OD values with control antigen. The use of specific antisera produced against viral antigens purified by CIEP techniques as capture antibodies in ELISA offers great possibilities of the attainment of more accurate information by diagnostic virological serology.

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LITERATURE CITED