Rapid Detection of Respiratory Syncytial Virus with a Monoclonal Antibody

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We developed an indirect fluorescent-antibody test employing a mouse monoclonal antibody directed against the nucleoprotein of RSV for rapid detection of respiratory syncytial virus (RSV). This test demonstrated distinctive fluorescent inclusions in HEp-2 cells infected with 24 RSV isolates collected during 6 previous years but not in cells infected with 13 other respiratory viruses. Examination of nasal cells of 100 infants with acute respiratory illness showed that the indirect fluorescent-antibody test employing the monoclonal antibody was 79% sensitive and 100% specific, as compared with the combination of both culturing and a similar indirect fluorescent-antibody test with commercial anti-RSV serum. This monoclonal antibody is an easily produced, well-characterized, sensitive, and specific reagent for the rapid detection of RSV antigen.

The rapid detection of respiratory syncytial virus (RSV) antigen in clinical specimens has been useful in diagnosing and limiting nosocomial spread of this important cause of bronchiolitis and pneumonia in infants and children (4, 10). In addition, the appropriate use of antiviral agents to treat RSV infections in the future will be assisted by readily available methods of rapid diagnosis. Although several investigators have developed direct or indirect fluorescent-antibody (IFA) tests for RSV antigen detection with various commercially available animal anti-RSV sera (1, 5, 7-9), these tests have not been widely used outside of research laboratories, in part because considerable experience is required to distinguish between viral and nonspecific background fluorescence. In addition, the lack of uniform standardization and precise characterization of commercially available antisera has made it difficult to compare results obtained with different lots of reagents. A monoclonal antibody against RSV may, therefore, be preferable to animal anti-RSV sera for use in antigen detection tests, since it would be a highly specific, well-characterized reagent which could easily be produced in large quantities.

We developed an IFA test employing a mouse monoclonal antibody (mIFA) directed against the nucleoprotein of RSV and compared this test with viral culturing and with a currently available commercial IFA (cIFA) test for the detection of RSV antigen in nasal secretions of infected children.

MATERIALS AND METHODS

Cells and virus. HEp-2 cells were maintained in minimal essential media with 5% fetal bovine serum. The Long strain of RSV (originally obtained from R. M. Chanock, National Institutes of Health, Bethesda, Md.) was grown to stock titer in HEp-2 cells and frozen at -70°C until use. A total of 24 wild RSV isolates from clinical specimens, collected between 1976 and 1981 (4 to 5 isolates per year), were initially isolated on HEp-2 cells and then frozen at -70°C until use. A total of 13 non-RSV isolates (parainfluenza virus types 1, 2, and 3, influenza A and B viruses, adenovirus, rhinovirus, herpes simplex virus, cytomegalovirus, and enterovirus [three types]) were obtained from frozen harvests of isolates from clinical specimens.

Specimens for IFA tests. Long strain RSV and 24 wild RSV isolates were inoculated onto HEp-2 cells. All other viruses were inoculated onto appropriate cell lines. When a cytopathic effect was seen, the monolayers were washed with phosphate-buffered saline (PBS), and the infected cells were scraped into 1 ml of PBS. The centrifuged pellets from these suspensions were mixed with a small volume of PBS to form a turbid suspension. One drop of this cell suspension was spread on a glass slide, allowed to air dry, and then fixed in cold acetone at 4°C for 10 min. Slides were stored at -20°C until used for IFA testing. Uninfected control cells were prepared in a similar fashion.

Nasal epithelial cells were obtained from 100 infants hospitalized with acute respiratory illness from November 1981 to February 1982. A moist, cotton-tipped applicator was used to swab the nasal mucosa. The applicator tip was placed in PBS and vortexed to suspend nasal epithelial cells. The cell suspension was

TABLE 1. Detection of RSV antigen in nasal cells of 100 hospitalized infants by an IFA test with a monoclonal antibody and by both an IFA test with commercial anti-RSV serum and viral isolation^a

mIFA assay	Viral isolation		cIFA assay		Positive by viral isolation or cIFA assay (n = 67)	Negative by viral isolation and cIFA assay (n = 33)
	Positive (n = 52)	Negative (n = 48)	Positive (n = 57)	Negative (n = 43)		
% Positive	85	19	82	14	79	0
% Negative	15	81	18	86	21	100

^a Numbers represent the percentages of specimens positive or negative by mIFA. The number of specimens that were positive or negative by viral isolation or cIFA are given in parentheses.

then centrifuged at 1,500 rpm for 10 min. The cells were washed three times with PBS, resuspended in a small volume of PBS to yield a slightly turbid suspension, and prepared for IFA testing as described above.

Monoclonal antibody production. Monoclonal antibodies were prepared to RSV proteins as previously described (E. E. Walsh and J. F. Hruska, J. Virol., in press). In brief, a 6-week-old CAF-1 mouse was immunized with 0.1 ml of RSV antigen (CF antigen, lot 39178; Microbiological Associates) in complete Freund adjuvant by intraperitoneal injection. After a booster inoculation 2 weeks later, the spleen cells were fused with nonsecretor x63/Ag8.653 BALB/c-derived myeloma cells. Anti-RSV hybridomas were identified by enzyme-linked immunosorbent assay, cloned twice by limiting dilution, and injected into pristane-primed mice; the antibody-rich ascites was then harvested. The RSV antibody used in the IFA test was determined to be an immunoglobulin G_{2a} by Ouchterlony double gel diffusion with specific anti-mouse immunoglobulin G antisera (Meloy Laboratories, Inc.), and its specificity against the 44,000-molecular-weight nucleoprotein of RSV was determined by radioimmunoprecipitation of [³⁵S]methionine-labeled RSV by polyacrylamide gel electrophoresis.

IFA testing. IFA tests were performed by standard methods (1). The smears were coated with either 20 µl of monoclonal ascitic fluid, diluted 1:125 in PBS (mIFA test), or 20 µl of commercial bovine anti-RSV serum, diluted 1:5 in PBS (cIFA test) (Burroughs Wellcome Co.). Slides were incubated in a humid chamber at 37°C for 1 h, washed three times in PBS (10 min each), and then air dried. The smears were then coated with 10 µl of fluorescein conjugate (Burroughs Wellcome) diluted in Evans blue (sheep anti-mouse immunoglobulin G diluted 1:75 or rabbit anti-bovine immunoglobulin G diluted 1:150 for the mIFA and cIFA tests, respectively). Slides were reincubated at 37°C for 45 min, washed three times in PBS, air dried, and examined under a UV microscope. All mIFA tests were read "blind" by one observer; a second observer independently made blind readings for all cIFA tests.

Viral isolation. At the same time that nasal swabs were obtained for antigen detection, a nasal wash was collected for viral isolation. These specimens were inoculated onto HEP-2 cells, human foreskin fibroblasts, and cynomolgous monkey kidney cells. The cultures were refed the following day and examined daily for 14 days for a cytopathic effect. All cultures on HEP-2 cells which did not demonstrate a cytopathic effect by day 7 were repassaged in HEP-2 cells. The development of typical syncytial plaques was scored as a positive result.

RESULTS

With the mIFA assay, distinctive fluorescent inclusions were seen within the cytoplasm of HEP-2 cells infected with all RSV isolates collected in previous years but not in cells infected with other viruses. For nasal cells of infants with respiratory illness, the mIFA test was 79% sensitive and 100% specific for the detection of RSV, as compared with the combination of both culturing and the cIFA test (Table 1). The IFA assays appeared to be more sensitive than viral culturing, in that 9 (19%) of 48 culture-negative specimens were positive by both mIFA and cIFA tests.

In RSV-infected HEP-2 and nasal cells, the pattern of fluorescence seen with the mIFA test (Fig. 1A) was different from the pattern observed with the cIFA assay of the same specimen (Fig. 1B). In particular, the cIFA assay showed a more diffuse intra- and pericytoplasmic fluorescence, whereas the mIFA assay showed very distinct intracytoplasmic inclusions which appeared in sharp contrast with the surrounding darker cytoplasm. The brilliance, color, and size of the intracytoplasmic inclusions were similar in both assays; the most notable and consistent difference was the distinctness of the inclusions in the mIFA test. Comparison of the mIFA and cIFA tests on the same specimens yielded very similar diagnostic results (Table 1), suggesting that either pattern of fluorescence was suitable for the identification of RSV-infected cells. Actually, each pattern had certain practical advantages and disadvantages. For example, the larger area of cellular fluorescence seen with the cIFA assay occasionally caused infected cells to be more easily visualized at low power. On the other hand, the pattern of prominent inclusions in sharp contrast with surrounding cytoplasm allowed easier differentiation of RSV-infected cells from nonspecific background fluorescence.

DISCUSSION

In this study, an IFA test with a mouse monoclonal antibody was highly sensitive and specific for the detection of RSV compared with both virus culturing and an IFA test with com-

FIG. 1. Nasal cells from an RSV-infected infant examined with an IFA test with a monoclonal antibody (A) and an IFA test with commercial anti-RSV serum (B). Note that there are more prominent inclusions and less background fluorescence in the IFA test with a monoclonal antibody.

mercially available bovine antiserum. Since there tended to be less nonspecific background fluorescence seen with the mIFA test, this assay might be easier to interpret by less experienced workers.

Of particular interest was the observation that nine nasal washes from which no viruses were isolated demonstrated immunofluorescence by both mIFA and cIFA assays, indicating that these assays may, at times, be more sensitive as well as more rapid than viral isolation. This finding, also noted in previous studies evaluating rapid antigen detection tests for RSV (2, 4-6), underscores a second potential advantage of immunoassays over viral isolation, namely, increased sensitivity as well as speed. In fact, IFA tests may generally be even more sensitive, relative to culturing, than our results suggest, since our rate of isolation of this labile virus probably exceeds that which is routinely achieved in many hospitals. In our study, a trained technician obtained nasal wash specimens (more reliable than a nasal swab for recovery of RSV [3]) and immediately carried them to the laboratory for inoculation into cultures of cells which were monitored for their sensitivity to RSV. This is contrary to clinical situations in which nasal swabs are obtained by a variety of

personnel and transported by a routine messenger to the laboratory, where additional delays may be encountered before inoculation of cell cultures for which sensitivity may not be monitored.

Since isolation of RSV may be difficult, as well as expensive, standardized antigen detection tests for RSV, as well as other viruses, are likely to play an increasingly important role in viral diagnosis in the future. The ease with which monoclonal antibodies can be produced may reduce the cost of these rapid viral diagnostic tests, and the high degree of antibody specificity may make the tests easier to interpret, thus facilitating their more widespread use.

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