JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1986, p. 383–384
0095-1137/86/020383-02$02.00/0

Vol. 23, No. 2

Comparison of Washed Nasopharyngeal Cells and Whole Nasal Secretions for Detection of Respiratory Syncytial Virus Antigens by Enzyme-Linked Immunosorbent Assay

R. MICHAEL HENDRY,[1,2]† * LAUREN T. PIERIK,[1] AND KENNETH McINTOSH[1,2]

Division of Infectious Diseases, The Children's Hospital,[1] and Department of Pediatrics, Harvard Medical School,[2] Boston, Massachusetts 02115

Received 26 August 1985/Accepted 28 October 85

We compared washed nasal epithelial cells with unfractionated nasal secretions as sources of respiratory syncytial virus (RSV) antigens in an indirect enzyme-linked immunosorbent assay (ELISA). Of 28 infants positive for RSV by virus isolation or direct immunofluorescence or both, 27 (96%) were positive by ELISA with whole nasal secretions, whereas only 19 (68%) were positive by ELISA with the matching washed-cell fractions. Furthermore, the ELISA absorbances obtained with nasal secretions were significantly greater than those seen with washed-cell fractions, indicating that whole nasal secretions contain relatively greater amounts of RSV antigens as measured by ELISA.

Solid-phase immunoassays for viral antigens in nasal secretions have been used for the diagnosis of respiratory syncytial virus (RSV) infections by ourselves (2, 6, 10) and others (4, 7, 8, 9, 11, 12). The sensitivity of these assays relative to culture and immunofluorescence (IF) has been a matter of concern. In an effort to increase sensitivity, we hypothesized that slow-speed centrifugation of cells and cellular elements might offer a simple method of concentrating antigens in nasal secretions while at the same time removing antibody which could bind soluble antigens and compete with antibodies used in the assay system. We therefore conducted this comparison of washed, centrifuged cell fractions with whole nasal secretions as samples for the measurement of RSV antigens by enzyme-linked immunosorbent assay (ELISA). The hypothesis proved to be wrong, and the results form the basis of this brief report.

Twenty-eight nasal secretions collected as nasopharyngeal (NP) aspirates and submitted to the Diagnostic Virology Laboratory of The Children's Hospital, Boston, Mass., during the 1981–1982 epidemic season were chosen for study. Specimens were obtained from inpatients, whose median age was 4.5 months (range, 0.5 to 26.0 months). Specimens were taken at the time of hospitalization and were transported to the laboratory on ice. Portions of the whole nasal secretions were processed for viral culture as previously described (10). Another portion of each nasal secretion was dispersed by probed sonication and frozen at −70°C for subsequent assay of total protein (1) and ELISA for RSV antigen (6, 10). A third portion was used to prepare washed NP cells by centrifugation (5). The washed, pelleted NP cells were suspended in sufficient phosphate-buffered saline (pH 7.2) to yield a visibly turbid suspension, of which part was fixed onto microscope slides and the remainder was frozen at −70°C and saved for ELISA for RSV antigen. IF staining was performed as described previously (10).

The ELISA for RSV was identical to that of our published method (6, 10), with the following modifications. Bovine anti-RSV antiserum (3) was used at a 1:500 dilution as the detector antibody, and goat anti-bovine peroxidase conjugate (Kirkegaard & Perry Laboratories) was used at a 1:1,000 dilution. Substrate (0.125 ml) was added to each well, and the reaction was terminated after 30 to 45 min by the addition of 0.025 ml of 3.5 M HCl per well. A_{490} was measured with a Biotek 307 IP spectrophotometer (Biotek Instruments, Burlington, Vt.). Cutoff values for negative specimens were computed as previously described (11). All specimens analyzed by ELISA were positive for RSV by isolation, IF, or both. Each specimen was assayed in duplicate by ELISA, and the cell-secretion pair from each patient was assayed on the same microtiter plate.

The results obtained by ELISA for washed cell-whole secretion specimen pairs with both IF staining and cell culture isolation are compared in Table 1. In general, those specimens that were ELISA positive as both whole secretions and washed-cell fractions were more likely to be positive by both IF staining and culture. On the other hand, the absence of ELISA-detectable antigen in washed-cell fractions does not correlate well with a negative result by IF staining, indicating that such staining is more sensitive than ELISA in detecting cell-associated antigens. Discordance between ELISA and infectivity indicates that noninfectious specimens may still contain detectable antigen (10). Table 1 also indicates that whole nasal secretions are superior to

TABLE 1. Comparison of culture and IF with ELISA for the detection of RSV in whole secretions and NP cells from infants with acute RSV infections

Result[a] of ELISA for RSV (secretion/cells)	No. of infants showing RSV results as tested by[b]:				
	IF		Culture		
	+	−	+	−	Unsatisfactory[c]
+/+	17	1	12	3	3
+/−	6	3	4	3	2
−/+	1	0	1	0	0

[a] +, Positive result; −, negative result.
[b] All specimens were positive by IF, culture, or both.
[c] Due to nonspecific degeneration of cells or bacterial-fungal overgrowth.

* Corresponding author.
† Present address: Division of Virology, Office of Biologics Research and Review, Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD 20205.

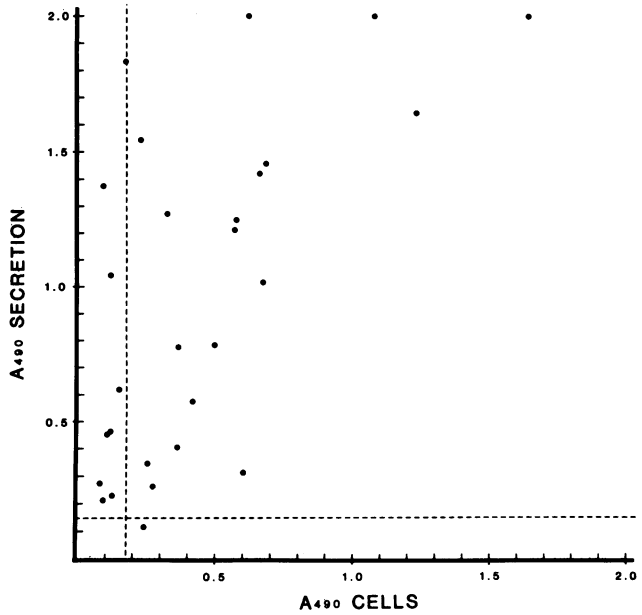

FIG. 1. Comparison of RSV antigen ELISA absorbance values obtained with either whole nasal secretions (*y* axis) or washed NP cells (*x* axis). Dotted lines indicate negative cutoff values.

washed-cell fractions for diagnosis of RSV infection by ELISA.

A quantitative comparison of whole nasal secretions and washed-cell fractions in an ELISA for RSV is shown in Fig. 1. In most cases, the highest ELISA absorbances were obtained with whole nasal secretions, and the mean difference in absorbance between secretions and cells was 0.511 absorbance units. This difference was statistically significant ($P = 0.0005$, Student's two-tailed t test). No significant correlations were seen between the ELISA absorbances for RSV obtained with either whole nasal secretions or washed-cell fractions with respect to total protein concentration, viral isolation, IF detection, or patient's age or sex.

The processing of whole nasal secretions for RSV antigen detection by ELISA can greatly affect the results obtained. A number of groups have used sonication in the presence of detergents as a means of releasing viral antigens (4, 11, 12). We have found that sonication can slightly increase the absorbances obtained by RSV ELISA but does not result in increased rates of positivity by ELISA (R. M. Hendry, unpublished results). Moreover, a single cycle of freezing and thawing should be sufficient to release the majority of cell-associated viral antigens. In a previous study, Sarkkinen et al. (12) reported that depletion of cells from RSV-positive whole nasal secretions resulted in slightly lower levels of antigen as measured by radioimmunoassay. They did not, however, directly compare whole nasal secretions with washed-cell fractions. We have found that whole nasal secretions are superior to washed-cell fractions as a source of RSV antigens as measured by ELISA. This finding would indicate that large amounts of extracellular virus or viral proteins are released from infected nasal mucosa during the course of RSV infection and should help optimize conditions for more sensitive immunochemical assays for the diagnosis of RSV infections.

We thank the staff of The Children's Hospital Diagnostic Virology Laboratory and Ellen Godfrey for technical assistance and Joy Field and Cathy Hobbs for preparation of the manuscript.

This work was supported, in part, by Public Health Service contract NOI-AI-92617 from the National Institutes of Health.

LITERATURE CITED

1. **Bradford, M. B.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
2. **Chao, R. K., M. Fishaut, J. D. Schwartzman, and K. McIntosh.** 1979. Detection of respiratory syncytial virus in nasal secretions from infants by enzyme-linked immunosorbent assay. J. Infect. Dis. **139**:483–486.
3. **Cranage, M. P., E. J. Stott, J. Nagington, and R. R. A. Coombs.** 1981. A reverse passive haemagglutination test for the detection of respiratory syncytial virus in nasal secretions from infants. J. Med. Virol. **98**:153–160.
4. **Ehrlicher, L., H. G. Hoffmann, and K. O. Habermehl.** 1984. Detection of respiratory virus antigens in nasopharyngeal secretions from patients with acute respiratory disease by radioimmunoassay and tissue culture isolation. Med. Microbiol. Immunol. **173**:37–44.
5. **Gardner, P. S., and J. McQuillan.** 1980. Rapid virus diagnosis, 2nd ed. Butterworth & Co., Ltd., London.
6. **Hendry, R. M., and K. McIntosh.** 1982. Enzyme-linked immunosorbent assay for detection of respiratory syncytial virus infection: development and description. J. Clin. Microbiol. **16**:324–328.
7. **Hornsleth, A., E. Brenøe, B. Friis, F. U. Knudsen, and P. Uldall.** 1981. Detection of respiratory syncytial virus in nasopharyngeal secretions by inhibition of enzyme-linked immunosorbent assay. J. Clin. Microbiol. **14**:510–515.
8. **Hornsleth, A., B. Friis, P. Andersen, and E. Brenøe.** 1982. Detection of respiratory syncytial virus in nasopharyngeal secretions by ELISA: comparison with fluorescent antibody technique. J. Med. Virol. **10**:273–281.
9. **Klein, B. S., F. R. Dollete, and R. H. Yolken.** 1982. The role of respiratory syncytial virus and other viral pathogens in acute otitis media. J. Pediatr. **101**:16–20.
10. **McIntosh, K., R. M. Hendry, M. L. Fahnestock, and L. T. Pierik.** 1982. Enzyme-linked immunosorbent assay for detection of respiratory syncytial virus infection: application to clinical samples. J. Clin. Microbiol. **16**:329–333.
11. **Meurman, O., H. Sarkkinen, D. Ruuskanen, P. Hanninen, and P. Halonen.** 1984. Diagnosis of respiratory synctial virus infection in children: comparison of viral antigen detection and serology. J. Med. Virol. **14**:61–65.
12. **Sarkkinen, H. K., P. E. Halonen, P. P. Arstila, and A. A. Salmi.** 1981. Detection of respiratory syncytial, parainfluenza type 2, and adenovirus antigens by radioimmunoassay and enzyme immunoassay on nasopharyngeal specimens from children with acute respiratory disease. J. Clin. Microbiol. **13**:258–265.