

Comparative Susceptibilities of Strain MRC-5 Human Embryonic Lung Fibroblast Cells and the Cooney Strain of Human Fetal Tonsil Cells for Isolation of Rhinoviruses from Clinical Specimens

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The comparative susceptibilities for rhinovirus isolation were determined for strain MRC-5 human embryonic lung fibroblast and human fetal tonsil cells. Samples from nasopharyngeal and throat swab clinical specimens were inoculated onto duplicate monolayers of each cell type. Of 105 rhinovirus-positive specimens, 78 (74%) were positive in MRC-5 cells, and 102 (97%) were positive in fetal tonsil cells ($P < 0.001$). For 75 specimens positive in both, the mean time to cytopathic effect development with standard deviation was 1 ± 2 days shorter in fetal tonsil cells ($P < 0.01$). The use of a second virus culture increased rhinovirus isolations by 10% compared with a single culture.

Rhinovirus infections are usually diagnosed by isolation of virus from respiratory secretions. Apart from some strains which will grow in monkey kidney cells, human rhinoviruses are usually isolated only in cells of human origin (4). Semicontinuous diploid cell strains from human embryonic lung are most widely used for primary virus isolation. Limited evidence suggests that human fetal tonsil cells, a diploid fibroblastic cell line, may be more sensitive than human embryonic lung fibroblasts for isolation of rhinovirus (1, 2). In a previous study, Fox and co-workers found that human fetal tonsil cells yielded rhinovirus isolates which had not been detected in strain WI-38 human embryonic lung fibroblasts (2). Although a continuous line of HeLa cells susceptible to rhinovirus (HeLa M) has also been used for primary isolation, this cell line was found to be less susceptible than fetal tonsil cells for recovery of rhinoviruses from known positive, frozen clinical specimens, even when blind passage of inoculated HeLa cells was performed (1). The current study was conducted to prospectively determine the relative sensitivity of human embryonic lung fibroblast (strain MRC-5) and fetal tonsil (Cooney strain) cells for isolation of rhinoviruses from fresh clinical specimens during a field study of intranasal interferon- $\alpha 2$. The results also allowed an assessment of the usefulness of inoculating duplicate monolayers and collecting a second specimen for isolation from persons with rhinovirus colds.

Fetal tonsil cells were kindly provided by Marion Cooney, University of Washington, Seattle, Wash., at passage 17, maintained in our laboratory, and used for virus isolation at passages 21 to 27. The cells were passaged at a 1:2 split ratio and grown in minimal essential medium containing 10% fetal bovine serum (HyClone Laboratories, Logan, Utah), 0.3 mg of glutamine per ml, and 20 μ g of vancomycin per ml, 50 μ g of gentamicin per ml, and 1 μ g of amphotericin B per ml. For virus isolation, confluent monolayers were grown in screw-capped roller tubes (16 by 125 mm) at 36°C in a 5.5% CO₂-air atmosphere. MRC-5 fibroblasts were purchased at passage 23 from M. A. Bioproducts, Walkersville, Md., in roller tubes and were refed upon receipt with the same growth medium used for the fetal tonsil cells.

Nasopharyngeal and throat swab specimens were taken from ill members of the families under study and transported in 4-ml volumes of chilled beef heart infusion broth containing 0.5% bovine serum albumin (Difco Laboratories, Detroit, Mich.), antimicrobial agents, and sheep antibody to recombinant interferon- $\alpha 2$ (kindly provided by Walter Protzman, Schering Corp., Kenilworth, N.J.) at a final concentration of 2,500 neutralizing units per ml (5). Specimens were maintained on ice during transport to the laboratory. Specimens received during the day were inoculated within 2 to 4 h of receipt, whereas those collected at night were held for 12 to 14 h at 4°C before inoculation. After vortexing and removal of the swabs, 0.2-ml volumes of the specimen were inoculated onto duplicate monolayers of MRC-5 and fetal tonsil cells and allowed to adsorb for 1 h at 36°C. Monolayers were then washed three times with phosphate-buffered saline (pH 7.2), and refed with minimal essential medium containing 10% fetal bovine serum and antimicrobial agents. Tubes were rotated on a roller drum apparatus at 33°C, and the monolayers were observed every other day for 10 days for the development of cytopathic effect. Isolates from monolayers with typical rhinovirus cytopathic effect were characterized as rhinoviruses by acid susceptibility testing in buffered glycine (pH 2.8).

Of 1,020 specimens collected for virus isolation, 105 (10.3%) yielded rhinoviruses. Of the rhinovirus-positive specimens, 78 (74.3%) were positive in MRC-5 fibroblasts, and 102 (97.1%) were positive in fetal tonsil cell monolayers ($P < 0.001$, Fisher's exact test) (Table 1). Of the 105 specimens, 75 were positive in both cell types, 27 were positive only in fetal tonsil cells, and 3 were positive only MRC-5 cell monolayers ($P < 0.001$). Overall, the use of fetal tonsil cells increased the number of rhinovirus isolates by 34.6%, as compared with the use of MRC-5 fibroblast cells alone.

For all specimens positive in MRC-5 fibroblast cells, the mean (range) time to development of cytopathic effect was 4 (2 to 11) days. For those positive in fetal tonsil cells, it was 4 (2 to 11) days. For the 75 specimens positive in both cell types, the mean time to development of rhinovirus cytopathic effect with standard deviation was 1 ± 2 days shorter in fetal tonsil cells than in MRC-5 fibroblast cell

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TABLE 1. Relative susceptibility of strain MRC-5 human embryonic lung fibroblast and human fetal tonsil cell monolayers for isolation of rhinoviruses and usefulness of inoculating duplicate monolayers

Specimen and cell type	No. of specimens positive for cytopathic effect in monolayers:			
	Either	Both	One	Neither
All positive ($n = 105$)				
MRC-5	78 ^a	62	16	27
Fetal tonsil	102 ^a	81	21	3
Positive in both cell types				
MRC-5	75	60	15	0
Fetal tonsil	75	67	8	0

^a $P < 0.001$ for MRC-5 cells versus fetal tonsil cells by Fisher's exact test.

monolayers ($P < 0.01$, paired t test). In 20 of the 75 specimens positive in both cell types, cytopathic effect was first detected in fetal tonsil cells, whereas cytopathic effect was detected in MRC-5 cells first in only three instances ($P < 0.001$).

Of the 75 specimens positive in both cell types, 15 yielded virus in only one of two inoculated MRC-5 fibroblast cell monolayers (20.0%), whereas only 8 (10.7%) specimens were positive in only one of two fetal tonsil monolayers ($P = 0.17$). Rhinovirus cytopathic effect in fetal tonsil cells tended to show larger foci than that in MRC-5 fibroblast cell monolayers, and the extent of cytopathic effect in parallel readings was significantly greater in fetal tonsil cells than in MRC-5 cells (Table 2). In 40 of 72 paired monolayers showing rhinovirus cytopathic effect, the extent of monolayer involvement was greater by at least 25% in the fetal tonsil cell monolayer than in the corresponding MRC-5 fibroblast cell monolayer; in seven instances it was greater in the MRC-5 cell monolayer; and in 25 instances the extent of involvement was comparable ($P < 0.001$).

The utility of inoculating duplicate monolayer tubes was assessed by determining the number of specimens positive in a particular cell type in which either both monolayers or only one of two monolayers was positive (Table 1). The majority of these specimens were positive in both inoculated monolayers. Of 78 specimens positive in MRC-5 fibroblasts, 16 (20.5%) were positive in only one of two inoculated monolayers. Similarly, of 102 specimens positive in fetal tonsil cells, 21 (20.6%) were positive in only one of two inoculated monolayers. If only one monolayer had been inoculated for either cell type, up to one-fifth of the isolates could have been missed.

The usefulness of collecting multiple specimens was assessed in 41 culture-positive patients who had specimens collected for isolation on two successive days. Of these patients, 7 yielded rhinovirus from day-1 specimen only, 4 yielded rhinovirus from the day-2 specimen only, and 30 yielded rhinovirus from both specimens. The use of a repeat culture increased the number of rhinovirus-positive patients by 10.8% relative to a single one.

In summary, the results indicated that the Cooney strain of human fetal tonsil cells was significantly more susceptible than MRC-5 human embryonic lung fibroblast cells for isolation of rhinovirus from clinical specimens. These results extend earlier findings indicating that fetal tonsil cells were more susceptible than strain WI-38 fibroblasts or rhinovirus-sensitive HeLa cells for isolation of rhinoviruses (1, 2). The

TABLE 2. Differences in the extent of involvement in 72 paired monolayers showing rhinovirus cytopathic effect

Difference in cytopathic effect scoring ^a	No. of monolayer pairs showing difference
-2	2
-1	5
0	25
+1	18
+2	15
+3	7

^a Extent of monolayer involvement was rated as 1 = 25%, 2 = 50%, 3 = 75%, and 4 = 100%, and the difference in same-day readings between the fetal tonsil and the MRC-5 fibroblast monolayers was calculated as the fetal tonsil cell reading minus the MRC-5 cell reading.

use of fetal tonsil cell monolayers increased the number of rhinovirus isolates by approximately 35% and significantly decreased the time to observable cytopathic effect, as compared with MRC-5 fibroblasts. The inoculation of duplicate monolayers also significantly increased recovery of rhinoviruses from clinical specimens as compared with the use of a single tube of either MRC-5 or fetal tonsil cells. Although other studies (3; A. Maiatico, O. Blahy, C. Forrer, and H. M. Friedman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, C36, p. 268) have found little additional yield from inoculating multiple tubes of the same type for other viruses, the results of the current study indicate that the common practice of inoculating duplicate monolayers provides an increased likelihood of rhinovirus recovery. The use of a second virus culture increased isolation rates by approximately 10%, compared with a single initial culture. For laboratories interested in respiratory virus isolation, the use of fetal tonsil cells offers significantly greater recovery rates of rhinoviruses from clinical specimens than commercially available cell strains.

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