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Localization of Rhinovirus Replication In Vitro With In Situ Hybridization

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To facilitate understanding of human rhinovirus (HRV) pathogenesis, methods were developed for detection of HRV infection in vitro using in situ hybridization (ISH). HRV-14 RNA probes and oligonucleotide probes representing conserved sequences in the 5'-non-translated region were labeled with ^{35}S and used to detect infected HeLa or WI-38 strain human embryonic lung cells in cytological preparations. ISH was shown to be specific for detection of HRV on a single-cell basis. Subsequently, in human nasal polyps infected in vitro, both oligonucleotide- and riboprobes produced a strong signal in association with ciliated epithelial cells. In human adenoids infected in vitro, a signal was observed in non-ciliated epithelial cells. This study shows that HRV replicates in ciliated cells in the epithelium of human nasal polyps infected in vitro, and the presence of viral RNA in non-ciliated cells of the human adenoid infected in vitro suggests that other cell types may also support rhinovirus replication.

KEY WORDS: picornavirus, RNA probes, oligonucleotide probes, ciliated epithelial cell

INTRODUCTION

Rhinoviruses, the most important cause of upper respiratory infections in humans [Gwaltney et al., 1989], are among the best characterized of all human pathogenic viruses. The molecular structure of human rhinovirus type 14 (HRV) has been resolved to the atomic level [Rossman et al., 1985]; the genome sequences of several serotypes have been determined [Skern et al., 1985; Hughes et al., 1988; Callahan et al., 1985; Duechler et al., 1987]; and the cell-receptor for approximately 90% of the over one hundred different serotypes has been identified [Greve et al., 1989; Staunton et al., 1989; Tomassini et al., 1989]. Nevertheless, the pathogenesis of rhinovirus infection remains unclear. Which cells of the respiratory mucosa are infected and where are they localized, how the virus disseminates through the epithelium of the upper air way, whether the infection extends into the lower respiratory tract, and how virus replication relates to symptom development remain incompletely defined.

No obvious histopathological abnormalities are detected in nasal mucosa biopsies from rhinovirus-infected persons by light microscopy [Winther et al., 1984]. Using HRV infection of modified organ cultures of nasal polyps and turbinates, Winther et al. [1990] did not observe any damage or cytopathic alterations in the epithelium, both in the fragment of human tissue and in the outgrowing monolayer. On the other hand, Turner et al. [1982] found that 2% of the cells sloughed in the nasal secretions of volunteers with induced colds were ciliated epithelial cells, while none of those cells were observed in the nasal secretions of normal controls. In the same study the presence of rhinovirus antigen was shown in association with sloughed ciliated cells using immunocytochemistry with a polyclonal antibody to HRV-39. Such observations suggest that rhinovirus replication occurs in ciliated epithelial cells. In spite of this evidence, rhinovirus replication in the respiratory epithelium has not been demonstrated, either in ciliated or nonciliated epithelial cells, while the cells are still attached to the epithelium. Previous work using immunofluorescence to localize infected cells in sections of organ cultures of human nasal polyps infected in vitro were inconclusive, mainly because of the presence of non-specific fluorescence [Hamory et al., 1977].

The feasibility of using ISH to identify the sites of rhinovirus replication in the respiratory epithelium was examined. The present study was undertaken to develop an in situ hybridization assay for detection of rhinovirus infection in cell cultures and to apply it to human organ cultures of nasal polyp and adenoids infected in vitro.

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MATERIALS AND METHODS

Viruses and Cells

Human rhinovirus types 14 (HRV-14) and 39 (HRV-39) were originally obtained from young adults with common colds, and used after serial passages in our laboratory. Rhinovirus Hanks (HRV-HH) is an untyped clinical isolate from our laboratory. The cell line HeLa R-19 was a gift from Dr. Richard J. Colonno (Merck, Sharp & Dohme, West Point, PA), and was serially passaged in growth medium consisting of Eagle's minimum essential medium (MEM) containing Earle's salts (Whittaker, Walkersville, MD), 10% serum, and antimicrobics. Human embryonic lung fibroblasts (strain WI-38) were purchased commercially (Whittaker, Walkersville, MD) and maintained in the same medium containing 2% serum. Adenovirus, herpes simplex virus type 1, respiratory syncytial virus, coxsackie virus A21, poliovirus type 1 were obtained from ATCC, and influenza virus A was a clinical isolate from our laboratory.

Cytological and Histological Specimens

Confluent monolayers of HeLa and WI-38 were infected with approximately $10^{4.5}$ $TCID_{50}$ of HRV-14, HRV-39, or HRV-HH in 25 cm^2 flasks, and incubated for 16 hours at 33°C. Cells were harvested when cytopathic effect (CPE) was detectable in approximately 30% of the monolayer, pelleted by centrifugation at 200 × g for 10 minutes at 4°C, washed with 1 × PBS, 10 Mm vanadyl ribonucleoside complex (VRC), fixed in 4% paraformaldehyde for 60 min, and washed 3 times with PBS plus 10 Mm VRC. Thereafter, cells were either incorporated in clots of human plasma or cytocentrifuged onto slides silanized following published procedures [Tourtellotte et al., 1987].

The cell pellet obtained from a 25 cm^2 flask was resuspended in 0.5 ml of human plasma to make a clot preparation, followed by addition of 20 μl of thrombin (Parke-Davis, Morris Plains, NJ), and mixed by agitation. The clot was fixed overnight in 4% paraformaldehyde, and paraffin-embedded. Five μm sections were picked up with silanized, freshly activated slides, air-dried, and stored in a desiccated box at room temperature.

For cytocentrifugation, the cell pellet was resuspended in PBS plus 10 mM VRC at a concentration of approximately 2×10^6 cells/ml. Forty μl of the cell suspension was loaded into each chamber of a plexiglass cytocentrifuge block, and centrifuged at 200 × g for 10 minutes in a Shandon Cytospin I (Shandon Southern Instruments, Sewickley, PA) [Maples, 1985]. After centrifugation, the slides were air-dried, fixed with 4% paraformaldehyde for 60 minutes, washed in PBS, dehydrated through an ethanol series, and stored in a desiccated box at 4°C for 2–3 weeks. Controls included non-infected cells, HRV-14 infected cells treated with RNase before hybridization, HRV-14 infected cells hybridized with a probe to coronavirus type 229E, and cells infected with adenovirus, herpes simplex, respiratory syncytial virus, or coxsackie virus A21.

Nasal polyps and adenoids were obtained from patients undergoing surgical removal of those tissues. Tissues were transported to the laboratory in Eagle's MEM, washed in 2 changes of the same medium to remove excess debris and blood clots, and, when necessary, cut to fragments of approximately 0.5 cm × 0.5 cm. A total of 12 fragments of nasal polyps obtained from 4 different patients and 15 fragments of adenoids obtained from 4 different patients were used in the study. The pieces of tissue were placed in wells of 24-well plates and exposed to 1.5 ml of suspensions of RV-14, or RV-39, at 10^5 $TCID_{50}$/ml in Eagle's MEM supplemented with 10% bovine fetal serum, for 3 hours, at 2% CO_2. Controls included non-infected fragments and fragments infected with RSV, influenza virus type A, and sections of rhinovirus infected tissue pre-treated with Rnase. For fragments infected with influenza A virus Eagle's MEM was used without supplementation with bovine serum. The inoculum was removed, the tissue fragments were washed three times with PBS, and then incubated with fresh medium, at 33°C. After 24 or 48 hours of incubation the medium was removed and the tissue was fixed overnight in 4% paraformaldehyde. The medium removed from the wells before the tissue fixation was titered using WI-38 cells just to confirm that infection had occurred. After paraffin-embedding, 5 μm sections were picked up on silanized slides. Before use, the slides were incubated at 60°C for 30 min, dewaxed by three 5-min washings in xylene, and the tissues were rehydrated through ethanol to PBS.

Preparation of Probes

RNA probes. A cDNA insert corresponding to nucleotides 443 to 3673 of the genome of HRV-14, cloned into pUC9 was a gift from Richard Colonno (from Merck Sharp and Dohme Research Laboratories, West Point, PA) [Callahan et al., 1985]. The insert was removed by digestion with *Pst I*, and sub-cloned into the plasmid pBS(−) (Stratagene, La Jolla, CA). Linearization of the recombinant plasmid with *Sal I* or *Sph I* allowed transcription of the insert with the RNA-polymerases T3 and T7, respectively. In an experiment in which RV-14 RNA was extracted, applied to a filter, and incubated with the 2 different radiolabeled transcripts, a much stronger signal was obtained with the transcripts generated by T3 RNA-polymerase, showing that those functioned as anti-sense probes for viral (+)strand RNA. An RNA transcription kit (Strategene, La Jolla, CA) was used to generate ^{35}S-labeled riboprobes with specific activities of $4 - 5 \times 10^8$ cpm/μg. Gel analysis showed that the radiolabeled transcripts ranged in size from approximately 0.1 to 2.4 kb, with predominance of fragments of approximately 0.8 kb. Alkaline digestion [Cox et al., 1984] for 20 min reduced the sizes of the fragments to approximately 0.2 kb. The probe was purified through RNAase-free Sephadex G-25 columns. RNA extracted from serial dilutions of

HRV-14 was hybridized in solution with HRV-14 antisense RNA probe followed by a RNase-protection assay with RNase A plus RNase T1 [Lee and Costlow, 1987]; the findings indicated that the sensitivity of the HRV-14 RNA probe was between 10^2 and 10^3 $TCID_{50}$ (data not shown).

Oligonucleotide probes. Two oligonucleotides were synthesized complementary to short sequences (nucleotides 451 to 467 and 547 to 562) in the 5′ non-translated region of the genome of HRV-14 [Stanway et al., 1984], which are conserved in picornaviruses [Bruce et al., 1989; Gama et al., 1989]. OL1 (451–467) was 5′GCATTCAGGGGCCGGAG3′, and OL2 (547–562) was 5′CGGACACCCAAAGTAG3′. A total of 500 ng of oligonucleotides were 3′-tailed with ^{35}S-dATP by terminal deoxynucleotide transferase (TDT), using the terminal labeling kit from Enzo (New York, NY). Non-incorporated nucleotides were removed from oligonucleotide probe preparations by 2 consecutive ethanol precipitations.

In Situ Hybridization

The slides were processed by serial treatment in the following solutions, all prepared with water treated with 0.025% (v/v) diethylpyrocarbonate (DEPC): 0.2 M HCl (20 min), PBS (5 min), H_2O (1 min), 0.125 mg/ml Pronase (CalBiochem, San Diego, CA) in 50 mM tris-HCl pH 7.5, 5 mM EDTA (10 min), 0.2% glycine in PBS (2 min), 4% (w/v) paraformaldehyde (30 min), PBS (5 min), 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0 (10 min), and PBS (5 min). At this point, selected slides were incubated with 150 μg/ml of bovine pancreatic RNase (CalBiochem, San Diego, CA) in 10 mM tris-HCl pH 7.5, 1 mM EDTA, for 1 hour at 37°C, followed by washing with active 0.1% DEPC-PBS for 15 min and with 4 changes of fresh PBS, for 1 min each, with vigorous shaking. The slides were dehydrated with ethanol, and air-dried. Those to be hybridized with RNA probe were covered with 100 μl of prehybridization mixture consisting of 50% freshly deionized formamide, 1 × hybridization buffer (0.3 M sodium chloride, 10 mM tris-HCl pH 6.8, 10 mM sodium phosphate, 5 mM EDTA), 4 × Denhardt's solution, 1 μg/μl heat-denatured salmon sperm DNA (ss-DNA), 1 μg/μl yeast tRNA, 10 mM DTT, 10 units of placental RNase inhibitor (Calbiochem, La Jolla, CA), in DEPC-H_2O, and incubated at 40°C, for 2–3 hours, in a humidified chamber. The slides were washed in 2 × hybridization buffer, dehydrated in ethanol diluted in 0.3 M ammonium acetate, and air-dried thoroughly. Hybridization mixture was the same as the pre-hybridization mixture, with the addition of 2–4 × 10^5 cpm of probe per section. Each slide was hybridized with 40 μl of mixture, covered by a silanized coverslip sealed around with rubber cement, and incubated overnight, at 40°C, in a sealed box humidified with DEPC-water. The rubber cement was removed and the slides were immersed in 1 × hybridization buffer, 20 mM DTT, 50% formamide, at 45°C to remove the coverslips, followed by washing in the same solution at 45°C for 90 min in a shaking water bath. The slides were washed in 0.5 M sodium chloride in TE plus 20 μg/ml bovine pancreatic RNase at 37°C for 30 min; followed by washing in 1 × SSC with 0.1% SDS and 10 mM DTT at 37°C for 30 min; dehydrated in ethanol diluted in 0.3 M ammonium acetate; and air-dried completely. For the slides to be hybridized with oligonucleotide probes, the procedure was the same as for RNA probes, with the following exceptions. Pre-hybridization and hybridization were both done at 37°C in 2 × SSC, 4 × Denhardt's solution, 20 mM VRC, 10 mM DTT, 1 μg/μl salmon sperm DNA and 1 μg/μl yeast tRNA. Post-hybridization washes consisted of two 10-min washes in 2 × SSC, 0.2% SDS, 10 mM DTT, at 40°C, and two 30-min washes in 2 × SSC, 0.2% SDS, 10 mM DTT, at room temperature.

Detection of ISH Signal

Ilford Nuclear Research Emulsion K5 (Polysciences, Warrington, PA) was melted at 42°C and diluted 1:1 with 0.6 M ammonium acetate. The slides were dipped twice in diluted emulsion, at 42°C for 2 or 3 seconds and allowed to dry upright in a humidified, light-proof box overnight. They were then transferred to another dry, light-proof box containing silica gel, and exposed at 4°C for 3 to 14 days. Positive and negative control slides were developed within 3, 7, and 14 days of exposure. When signal to noise ratio was judged to be adequate, all slides were developed with Kodak D-19 developer for 3 minutes at room temperature, followed by a brief wash in water, fixed for 1 min in Kodak rapid fixer, and washed in tap water for 5 min. The slides were stained with toluidine blue or hematoxylin/eosin, following standard procedures [Sheehan and Hrapchak, 1980], and mounted with permount (Fisher Scientific, Springfield, NJ). Definite positive signal of ISH was considered to be an accumulation of silver grains observed in clear association with an underlying visible cell. All slide readings were under masked conditions. Counting of silver grains was done using the software "GrnCnt" and a Gould IP8500 workstation connected to a mainframe computer PDP 11/70, at the Biomedical Image Processing Center of the University of Virginia.

RESULTS

Specificity and Sensitivity of the In Situ Hybridization With RNA and Oligonucleotide Probes for HRV RNA

The specificity of the in situ hybridization method was tested initially in HeLa and WI-38 cells with the RNA probe for (+)strand HRV-14 RNA. No hybridization signal was observed in 102 negative control preparations (Table I and Fig. 1a). In contrast, a signal was observed in all of 51 preparations of HeLa or WI-38 cells infected with HRV-14. The autoradiographic background was negligible, and the morphology of the cells was well preserved. Cells harboring rhinovirus were clearly distinguishable by the accumulation of silver grains (Fig. 1b). The percentage of cells showing

TABLE 1. Specificity of In Situ Hybridization With HRV-14 RNA Probe

	Number of cell preparations[a]						
	Uninfected (HeLa)	Uninfected (WI-38)	Cox (WI-38)	RSV (HeLa)	HSV (WI-38)	Adeno (HeLa)	RNase
Signal present	0	0	0	0	0	0	0
Total tested	49	30	8	2	5	2	6

Abbreviations: Cox = infected with coxsackie virus type A21, RSV = respiratory syncytial virus, HSV = herpes simplex virus, Adeno = adenovirus, RNase = cells infected with HRV-14 and treated with RNase prior to hybridization.
[a]The infecting virus (cell culture type) are indicated.

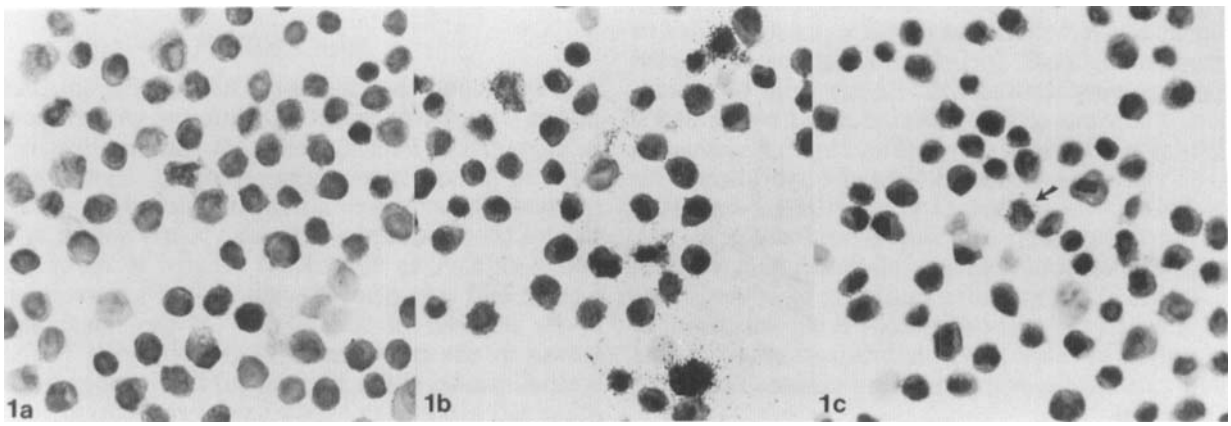

Fig. 1. In situ hybridization of HeLa cells cytocentrifuged onto microscope slides with ^{35}S-labeled RNA probe for HRV-14 (365 ×, HE staining). **a:** Uninfected cells; **b:** cells infected with HRV-14 and hybridized with RNA probe specific for (+) strand viral RNA; **c:** cells infected with HRV-14 and hybridized with RNA probe specific for (−)strand viral RNA (the arrow points to an infected cell). (Exposure time: 5 days.)

ISH signal was established in 45 preparations obtained in 13 different experiments. The mean (±S.D.) fraction of cells showing definite ISH signal was 11.6 (±9.7) percent. Three preparations of cells infected with HRV-14, and hybridized with the sense RNA probe, showed definite signal, but the intensity of the signal, as determined by the number of grains per cell, was approximately 1/10th that obtained with the anti-sense RNA probe (Fig. 1c). When the grains associated with 30 positive cells per preparation were counted, the results were 285(±131) grains/cell for HRV-14 infected cells hybridized with anti-sense RNA probe, and 32(±22) grains/cell for HRV-14-infected cells hybridized with sense RNA probe ($P < 0.0001$, t-test). The numbers of grains associated with 30 randomly selected cells in the negative control slides were 0.9(±0.9) for HRV-14 infected cells treated with RNase, and 0.5(±0.6) for non-infected cells. Two slides of HRV-14-infected cells hybridized with a RNA probe to coronavirus type 229E did not show any signal. At the stringency of hybridization and post-hybridization washes, cells infected with other serotypes of rhinovirus (13 preparations of HRV-39 and 13 preparations of HRV-HH infected cells) did not show signal with HRV-14 RNA probe.

When ^{35}S-labeled oligonucleotide probes were used, hybridization signal was observed with 7 of 7 slides of cells infected with HRV-14, 8 of 8 slides of cells infected with HRV-39, and 3 of 3 slides of cells infected with HRV-HH, whereas no hybridization was detected in 14 preparations of non-infected cells, or in 4 slides of cells infected with HRV-14 and treated with RNAase prior to hybridization. Therefore, the observation of silver grains after hybridization with HRV-14 ^{35}S-labeled RNA probe correlated with the presence of HRV-14, indicating that in situ hybridization was specific for HRV-14 nucleic acid, while the oligonucleotide probes hybridized with different rhinovirus serotypes.

Localization of Rhinovirus Replication in Cultures of Nasal Polyp Fragments

In 10 out of 11 sections obtained from 4 different fragments of nasal polyps infected with HRV-14 overnight, hybridization with anti-sense RNA probe showed that signal was very well localized and related to focal areas of the respiratory epithelium. In those areas, silver grains outlined the epithelial border and distinctly concentrated on discrete ciliated epithelial cells (Fig. 2a). Hybridization signal was not observed in association with any other cell type. Hybridization signal was also detected in 7 of 9 sections obtained from 4 different fragments of nasal polyps infected with HRV-14 and in 3 of 3 sections from 3 different fragments of nasal polyps infected with HRV-39 hybridized with oligonucleotide probes. The background was negligible, and no hybridization signal was documented in the negative controls, including 5 sections of infected polyps that were treated with RNase prior to hybridization, 4 sections of RSV-infected nasal polyp, and 6 sections of non-infected polyps (Fig. 2b). In 3 sections of nasal polyps infected with HRV-14 and hybridized with sense RNA probe, although rarer ciliated epithelial cells were identified exhibiting hybridization signal, this signal was well-defined, cell-associated, without the presence of the epithelial outlining by silver grains observed when the anti-sense RNA probe was used. In some regions of those polyps hybridization signal was in clear association with sloughing ciliated epithelial cells (Fig. 2c).

Localization of Rhinovirus Replication in Cultures of Adenoid Fragments

The adenoid fragments used consisted of stratified squamous epithelium with only occasional ciliated epithelial cells, and underlying lymphoid follicles. In 8 of 9 sections obtained from 3 fragments of adenoid infected with HRV-14 as well as in 4 of 4 fragments infected with HRV-39, hybridization with RNA or oligonucleotide probes produced signal in association with non-ciliated cells of the epithelium (Fig. 3a). Weaker signal associated with the same kind of cell was obtained with sense RNA probe. A signal was not observed in the lymphoid tissue in the lamina propria or within the lymphoid follicles. A signal was also not detected in association with ciliated cells of the adenoid epithelium. Signal was absent in sections of 6 different fragments of non-infected adenoid, in 2 sections of RSV-infected adenoid, and in 2 sections of influenza A virus-infected adenoid. Signal was abolished in 5 sections of adenoid infected with either HRV-14 or HRV-39 that were treated with RNAase prior to hybridization (Fig. 3b).

DISCUSSION

It was found that ISH with RNA or oligonucleotide probes was able to detect cells infected with rhinovirus in vitro and to identify cells that were replicating the virus in human respiratory epithelial tissues infected in vitro. Under the conditions employed for the infection of the fragments of human nasal polyps or adenoids, rhinovirus replication seemed to occur rather focally, and only ciliated epithelial cells were observed to be infected in the mucosa of the nasal polyps. However, the presence of viral RNA in non-ciliated epithelial cells of the pharyngeal tonsil suggested that other cell kinds may support viral replication.

The results of the experiments done with infected cell monolayers showed that ISH specifically detected viral infection in individual cells, allowing for distinction on a single-cell basis, and that RV-14 RNA probe was serotype-specific. The specificity of this probe in ISH studies confirms our earlier observations in dot-blot experiments (data not shown). This observation indicates dissimilarity of rhinovirus type 14 to the other

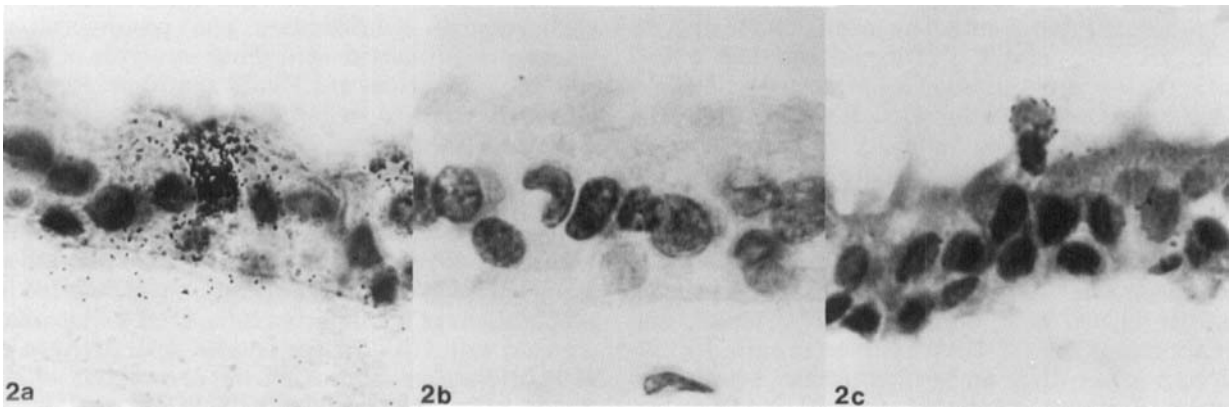

Fig. 2. In situ hybridization of sections of human nasal polyps with ^{35}S-labeled RNA probes for HRV-14 (960 ×, HE staining). **a:** Signal associated with a discrete ciliated epithelial cell after hybridization of HRV-14-infected nasal polyp with probe specific for (+)strand viral RNA; **b:** ciliated epithelium of uninfected nasal polyp hybridized with probe specific for (+)strand viral RNA; **c:** signal associated with a sloughing ciliated epithelial cell after hybridization of HRV-14-infected nasal polyp with probe specific for (−)strand viral RNA. (Exposure time: 5 days.)

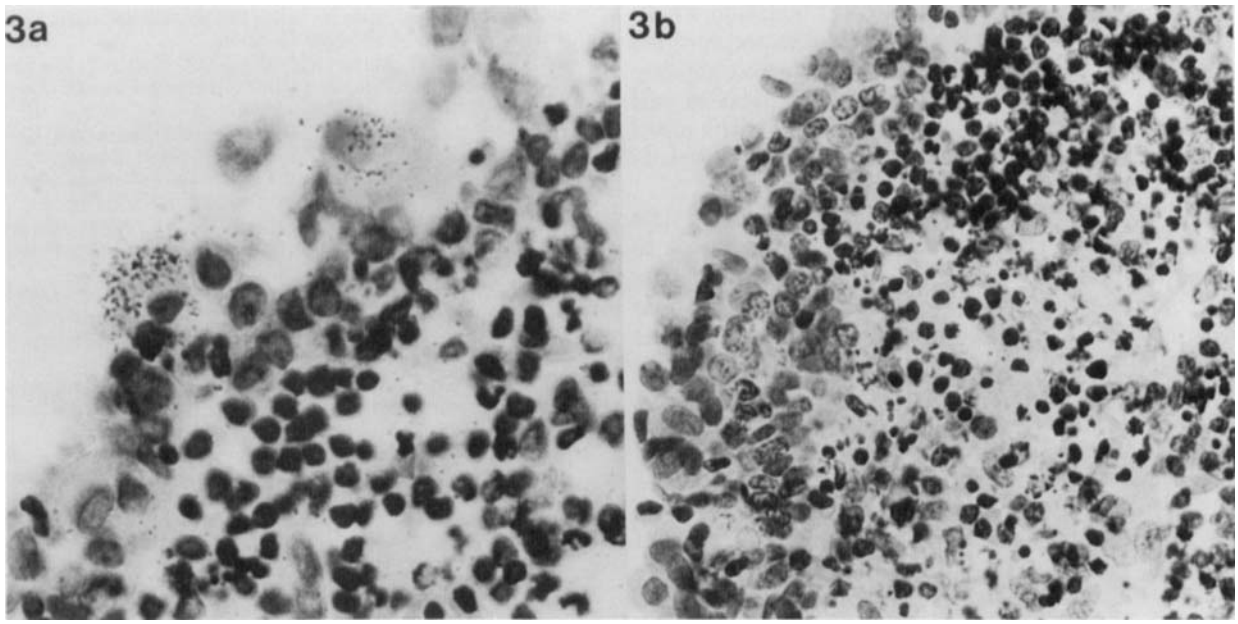

Fig. 3. In situ hybridization of sections of human adenoid with ^{35}S-labeled RNA probe for HRV-14 (HE staining). **a:** Signal associated with non-ciliated epithelial cells of HRV-14-infected adenoid hybridized with probe specific for (+)strand viral RNA (750 ×); **b:** non-ciliated epithelium of uninfected adenoid hybridized with probe specific for (+)strand viral RNA (500 ×). (Exposure time: 5 days.)

rhinovirus serotypes and is in agreement with results of nucleotide alignments in the P1 region [Palmenberg, 1989], which corresponds to approximately 80% of our probe. In contrast, the oligonucleotide probes demonstrated cross-reactivity with different rhinovirus serotypes, a finding predicted from earlier studies using slot-blot hybridization [Bruce et al., 1989].

The localization of rhinovirus replication in the respiratory tract has been difficult to investigate for several reasons. Firstly, rhinovirus replication in the human nasal epithelium seems to occur focally, rather than uniformly dispersed through the nasal epithelium [Winther et al., 1986]. This may create problems in sampling when random biopsies of the nasal mucosa are obtained. Secondly, the nasal secretions of patients with common colds contain cell-free virions which are free to adhere to the receptors present on the sloughed cells. This is a potential confounder of the distinction between cells with adherent virus and genuinely infected ones.

In this study, sections of nasal polyps epithelium were tested, as opposed to whole cell preparations. Therefore, the signal associated with all the extension of a given epithelial cell can reasonably be assumed as indicative of viral replication in that cell, and not merely adhesion of virions to the cell membrane. Moreover, the signal obtained with the sense RNA probe, which is able to detect only the replicative intermediates (−strand RNA), is definite evidence that replication takes place in ciliated epithelial cells. This finding confirms the study of Turner et al. [1982], in which the shedding of ciliated epithelial cells in the expelled nasal mucus of patients was quantitated, and the presence of viral antigens in the extruded cells was determined by immunocytochemistry with a polyclonal antiserum raised in guinea pigs against the homologous rhinovirus serotype. The staining of extruded ciliated epithelial cells suggests that those cells harbor viral replication; however, the staining of structural proteins of virions adhered to the cells cannot be ruled out. Moreover, the relative non-specificity of the immunoperoxidase staining was demonstrated in another study conducted by Turner et al. [1984], in which staining was observed in 8% of the samples obtained from 5 sham-inoculated volunteers.

The results of in situ hybridization with both kinds of probes in sections of nasal polyps infected in vitro for 24 or 48 hours, demonstrated that infection is not uniform throughout the mucosa, at least at those two points in time. It was also demonstrated that focal hybridization signal was in clear association with discrete ciliated cells, many of them sloughing from an apparently healthy epithelium. These in vitro findings suggest that only a small number of ciliated cells of the nasal epithelium are infected, in spite of the large inoculum used, as compared to the inoculum size that is supposed to occur in natural infections. This is in keeping with previous in vivo studies, which employed viral culture of different sites of the nasal epithelium [Winther et al., 1986]. Why the infection appears to be so focal in

nature is an interesting and important question which remains to be answered. This focal appearance, coupled with an apparently infrequent dispersal to neighboring cells, might be the explanation for the minimal cell damage seen in studies by light microscopy of the nasal mucosa of infected individuals shedding rhinovirus [Winther et al., 1984].

Nasopharyngeal tonsils, and other lymphoid tissues along the respiratory and digestive tracts, are able to perform the transport of foreign material from the exterior to the lymphoid cells, by the action of antigen presenting cells located in the epithelium overlying the lymphoid follicles [Richtsmeier and Shikhani, 1987]. In the case of viral infections, these cells may represent the pathway for entry in the host, leading to the first contact between the virus and the immune system, as shown for reovirus and M cells of the Peyer's patches of the intestine [Wolf et al., 1981]. In the present study, rhinovirus RNA was detected in cells of the adenoid epithelium. Based on their morphological appearance in HE counterstained sections, we can only say that they were not ciliated cells. While combined ISH/immunohistochemistry studies in order to further determine whether those cells are antigen presenting cells are necessary, this finding suggests that non-ciliated cells may support rhinovirus replication.

In summary, an ISH procedure which can identify rhinovirus-infected cells in cytological samples and paraffin-embedded tissue fragments is described. While ciliated epithelial cells seem to be the site of rhinovirus replication in nasal polyps infected in vitro with rhinovirus, the evidence for rhinovirus replication in non-ciliated cells of the adenoid is suggestive that other cell types may also support rhinovirus replication.

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