Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Virological Methods 125 (2005) 41–47
Journal of Virological Methods
www.elsevier.com/locate/jviromet

Development of rhinovirus study model using organ culture of turbinate mucosa

Yong Ju Jang*, Si Hyeong Lee, Hyon-Ja Kwon, Yoo-Sam Chung, Bong-Jae Lee

Department of Otolaryngolgy, Asan Medical Center, University of Ulsan College of Medicine, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Received 12 October 2004; received in revised form 15 December 2004; accepted 16 December 2004

Abstract

To better understand the pathophysiology of rhinovirus (RV) infection, a development of a study model using organ culture of turbinate mucosa was sought. Inferior turbinate mucosal tissues were cultured using air–liquid interface methods, on a support of gelfoam soaked in culture media. RV-16 was applied to the mucosal surface and washed off, and histological changes were evaluated. The success of RV infection was assayed by semi-nested RT-PCR of the mucosal surface fluid taken 48 h after incubation. Intracellular RVs were visualized by in situ hybridization (ISH). Secretion of the cytokines, IL-6 and IL-8, into the culture media was quantitated by ELISA. After 7 days of culture, the turbinate mucosae did not show significant damage. A PCR product indicating successful RV infection was detected in 5 out of 10 mucosal tissues. ISH showed a very small number of positively stained cells focally located in the epithelial layer. In the beginning 24 h after infection, secretion of IL-6 and IL-8 into the culture media of infected mucosae was significantly greater than into the media of control mucosae. Our results indicate that the air–liquid interface organ culture of turbinate mucosa could serve as an acceptable in vitro model for studying RV infection.

Keywords: Rhinovirus; Study model; Organ culture; Turbinate

1. Introduction

Common pathogens associated with viral upper respiratory infection (VRI) include rhinovirus (RV), coronavirus, influenza virus, parainfluenza virus, and adenovirus (Gwaltney et al., 1996). Among these, RV is the most frequent cause of the common colds in adults and children, being responsible for 50 to 60% of cases (Greenberg, 2003). VRI due to RV is clinically significant in otolaryngologic practice since it commonly precedes and predisposes to bacterial rhinosinusitis and otitis media (Greenberg, 2003; Pitkaranta et al., 2001).

RV is a nonenveloped 30 nm RNA-virus belonging to the Picornaviridae family (Greenberg, 2003). Nasal and nasopharyngeal epithelial cells are the primary targets of RV infection. These cells express on their surface the intercellular adhesion molecule (ICAM)-1 or low density lipoprotein receptor, the sites of RV attachment (Casasnovas and Springer, 1994). Following attachment of RV to its receptor, the entire virus is translocated across the epithelial cell membrane and uncoated releasing viral RNA into the cytoplasm for replication. RV infection triggers epithelial cell production of cytokines such as interleukin (IL)-6 and IL-8, which function as a neutrophil chemoattractants and are responsible for cold symptoms (Zhu et al., 1997). RV infection has been shown to stimulate the elaboration of other cytokines, including IL-1, TNF-α, RANTES, MIP, and IL-11, which also contribute to the cascade of inflammatory reactions (Terajima et al., 1997; Yuta et al., 1998).

Despite the obvious medical importance of RV infection, its pathophysiology is not thoroughly understood. In addition, the pathogenic mechanisms by which RV induces sinusitis are not clear (van Cauwenberge and Ingels, 1996).

* Corresponding author. Tel.: +82 2 3010 3712; fax: +82 2 489 2773.
E-mail address: jangyj@amc.seoul.kr (Y.J. Jang).

0166-0934/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2004.12.004

To better understand the pathophysiology of RV infection, an ideal experimental model is of critical importance. However, a practical animal model for studying RV infection is not yet available. Therefore, to date, studies of RV infection have mostly been performed by in vivo challenge of volunteers using nasal lavage fluid or infected mucosal tissue (Yuta et al., 1998) and by primary culture of tracheal and bronchial epithelial cells or culture of transformed cell lines (Zhu et al., 1997; Sethi et al., 1997). However, each of these methods has limitations. The in vivo challenge method is associated with ethical consideration, which precludes certain types of experimental work in humans. Culture of isolated epithelial cells probably reflects only a partial picture of the inflammatory response in mucosae, inasmuch as inflammatory cells, such as mast cells, eosinophils, macrophages, lymphocytes, endothelial cells, and glandular elements, are also involved in the entire RV-induced inflammatory process (Schierhorn et al., 1999). Furthermore, primary culture of tracheal epithelial cells or cell lines of the lower respiratory tract does not fully reflect the exact physiological changes occurring in the nasal cavity or nasopharynx mucosa, the primary site of RV infection and an area of interest in the pathogenesis of RV-induced sinusitis.

Therefore, there is a need for human in vitro models that are safe, reliable, and physiologically relevant. In this study, in attempting to overcome the limitations of the conventional study methods, the authors sought to develop a new model of RV infection. Since the upper airway, especially the nose, is a major target of RV infection (van Cauwenberge and Ingels, 1996), it is hypothesized that organ culture models of turbinate mucosae may be physiologically relevant. Here the authors describe the development of an air-interface organ culture system for growing RVs in turbinate mucosae, which are readily available from various nasal surgical procedures. The feasibility of this model was examined by confirmation of RV infection using semi-nested RT-PCR and in situ hybridization (ISH), and by measuring IL-6 and IL-8 secretion as markers of the inflammatory response to RV infection.

2. Materials and methods

2.1. Subjects

Inferior turbinate mucosae were harvested from 20 patients, with a mean age of 32 years (range: 17–52 years), who underwent septoturbinoplasty for the treatment of symptomatic nasal septal deviation. Turbinates from five patients were used for histologic evaluation of the effect of our culture methods on their integrity. Tissues from 10 patients were subjected to RV-infection and subsequent semi-nested RT-PCR and ISH. Turbinate tissues from five patients were infected with RV, and their culture media was used to assay cytokine secretion. Subjects who with a history of URI in the previous 2 months, medication with antihistamines or antibiotics in the previous 1 month, or paranasal sinusitis in the previous 6 months were excluded. Subjects were routinely checked with sinus X-ray to confirm the absence of sinus pathologies. The presence of allergy was excluded by skin prick testing with 11 common aeroallergens (Bencard TM, Bradford, England). All subjects signed informed consent forms and the study design was approved by the institutional review board of Asan Medical Center.

2.2. Viral stock preparation

RV-16 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Additional viral stocks were prepared by infection at 33 °C of HeLa cells (ATCC), which have a low-input multiplicity of infection (MOI). When the infection was notably advanced, as evidenced by cytopathic effects, the cell supernatants were harvested, the cells were disrupted by freezing and thawing, and the debris was pelleted by low-speed centrifugation. Aliquots of clarified supernatants were frozen at −70 °C after determining their viral titers.

2.3. Organ culture and RV infection

Inferior turbinate mucosal tissues were washed three times in HEPES buffer to remove blood cells and mucus, divided into four small pieces with a 4 mm biopsy punch (Stiefel, Offenbach, Germany), and placed in minimal essential medium (MEM) (GIBCO, Grand Island, NY), supplemented with 2 mM L-glutamine, 20 mM HEPES, penicillin (100 U/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml) for 4 h at 4 °C. Each tissue sample was implanted at the air–liquid interface, with the epithelium up and the submucosa down, on a 5 mm × 5 mm support of gelfoam (Johnson & Johnson, Gargrave, UK), prehydrated for at least 24 h with culture medium (Fig. 1). Each fragment was placed in a well of a 24 well plates (Nunclon; Nunc, Roskilde, Denmark) along with 1.5 ml culture medium, so that the epithelium was above the liquid phase. To the top of each was applied 30 μl of RV-16 (10^5 $TCID_{50}$ U/ml) for 4 h, following which the samples were washed four times with phosphate buffered saline (PBS). The RV-infected mucosae were transferred to a second set of identical plates and incubated for additional 48 h at 33 °C.

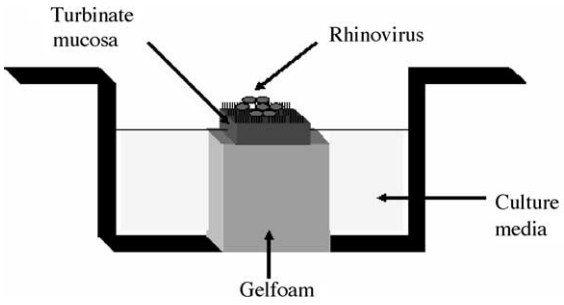

Fig. 1. Schematic illustration of the organ culture system used in this study. A mucosal piece is placed onto gelfoam support that has been soaked in culture medium. Rhinovirus is applied to the apical surface of the turbinate mucosa.

2.4. Histology

To evaluate the histological changes in turbinate mucosae after organ culture, five mucosal tissues were cultured for 7 days, as above, but without RV infection. Samples were stained before and after culture with hematoxylin–eosin (H&E) and examined by light microscopy at 200× magnification. Histologic change was evaluated in a blinded manner from coded slides, with the evaluators having no information about the organ culture.

2.5. Semi-nested RT-PCR for RV detection

2.5.1. Preparation of RV RNA and picornavirus RT-PCR

RNA was extracted from mucosal surface fluid using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

2.5.2. Picornavirus RT-PCR

Picornavirus RNA was reverse transcribed and amplified using the primers OL26 (5′-GCACTTCTGTTTCCCC-3′) and OL27 (5′-CGGACACCCAAAGTAG-3′) and the Invitrogen Superscript One-Step RT-PCR with PLATINUM *Taq* kit. (Invitrogen, Gaithersburg, MD, USA) Each PCR mixture contained 2× reaction buffer, 0.4 mM dNTP, 2.4 mM MgSO4, 0.5 μl of each primer, 0.4 μl of Platinum *Taq* mix, and 8.8 μl of the RNA template in a final volume of 20 μl. The PCR reaction was conducted in a thermal cycler (Perkin-Elmer 9600, Minneapolis, Minnesota, USA) programmed for initial cDNA synthesis and pre-denaturation (50 °C for 1 h and 94 °C for 2 min), followed by 36 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C, and extension for 30 s at 68 °C. The PCR products generated by this protocol were 388 base pair (bp) amplificon from a region of the 5′-noncoding region of the picornavirus genome.

2.5.3. Semi-nested RT-PCR

The picornavirus PCR product was then amplified using the primers OL26 and JWA-1b (5′-CATTCAGGGGCCGGAGGA-3′). The PCR mixture contained 2× reaction buffer 0.4 mM dNTP, 2.4 mM MgSO4, 0.4 μl of each primer, 1 U of AmliTAq (Applied Biosystems, Forster City, CA) and 5 μl of the RT-PCR product in a final volume of 20 μl. The amplification protocol consisted of 36 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 55 °C and extension for 30 s at 68 °C, followed by a final extension for 10 min at 72 °C. The PCR product generated by this protocol was 292 bp amplicon within the original amplificon.

2.6. In situ hybridization (ISH)

2.6.1. Preparation of slides and prehybridization

Mucosal tissues infected for 4 h with RV followed by incubation for 48 h without RV were fixed with optimal cutting temperature compound solution and sliced into 4 μm thick slices, which were mounted on the glass slide and dried for 10 min at 37 °C. The slides were incubated in acetone for 5 min, washed with running water and stored at −70 °C. They were subsequently bought to room temperature and incubated with 0.1% DEPC for 5 min and with 100 mM glycerine for 10 min. The slides were washed with PBS for 5 min, incubated with 0.3% Triton X-100 for 15 min and washed with PBS for 5 min. The slides were incubated with protease K (Oncogene, Boston, MA) for 30 min at 37 °C, washed with PBS for 5 min, fixed with 4% paraformaldehyde for 5 min and washed with PBS for 5 min.

2.6.2. Preparation of probes

A mixture containing 4 μl of 5× reaction buffer (1 M potassium cacodylate, 125 mM Tris–HCl, 1.25 mg/ml bovine serum albumin, pH 6.6), 4 μl of 25 mM $CoCl_2$, 1 μl of 1 mM digoxigenin-11-ddUTP, 1 μl of terminal transferase (Roche, Manheim, Germany) and 100 mol oligonucleotide probe in a final volume of 20 μl was centrifuged and mixed with 2 μl of 0.2 M EDTA (pH 8.0) on ice. The oligonucleotide probes used were PB4 (5′-CAGGGGCCGAGGACTCAAGATGAGCACACGCGGCTC-3′) and PB5 (5′-TGCAGGCAGCCACGCAGGCTAGAACTCCGTCGCCG-3′).

2.6.3. Hybridization

The probe was mixed with hybridization solution (2× SSC, 1× Denhardt's solution, 50 mM phosphate buffer pH 7.0, 50 mM DTT, 250 μg/ml yeast tRNA, 5 μg/ml polydeoxyadenylic acid, 100 μg/ml polyadenylic acid, 50% formamide, 10% dextran sulfate, 500 μg/ml denatured and $_{SS}$DNA) and 50 μl of this mixture was dropped onto the mucosal tissue on slide. Each was covered with a cover glass and incubated for more than 15 h at 37 °C. Each slide was immersed in 2× SSC to remove the cover glass, washed twice with 2× SSC for 15 min each and twice with 1× SSC for 5 min each. Each slide was washed twice with buffer I (100 mM Tris–HCl pH 7.5, 150 mM NaCl) for 10 min each while shaking and incubated in buffer II (100 mM Tris–HCl pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 1% blocking reagent) for 30 min. Onto each slide was placed anti-DIG-alkaline phosphatase (Fab fragment), diluted 1:100 in buffer II for 2 h at room temperature and incubated in buffer III (100 mM Tris–HCl, pH 7.5, 100 mM NaCl, 50 mM MgCl2) for 10 min. A mixture of 4.5 μl nitroblue tetrazolium (NBT) and 3.5 μl 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) in 1 ml buffer III was placed onto each slide, and the slides were covered and incubated for 2–24 h. Each slide was examined by light microscopy at ×200 magnification. All ISH results were evaluated in a blinded manner from coded slides, with the evaluators having no information about the RT-PCR results. Positive hybridization signal was defined by visible cytoplasmic reactivity.

2.7. Cytokine quantification

Five mucosal tissues were divided into five pieces each. One was used as control, being incubated for 24 h without RV application. The remaining four tissues were infected with RV for 4 h, followed by incubation for 2, 4, 8, and 24 h. Culture media were stored at 70 °C until assayed. IL-6 and IL-8 levels in basolateral culture media were assayed using ELISA kits (Biosource Co., Nivelles, Belgium) according to the manufacturer's protocol.

2.8. Statistical analysis

Differences in cytokine concentrations between control and experimental cultures were analyzed using Wilcoxon signed rank test. A p value less than 0.05 was defined as being statistically significant.

3. Results

3.1. Histological changes in turbinate mucosae after organ culture

Following organ culture for 7 days, the pseudostratified columnar epithelium of the turbinate was well preserved without obvious abnormalities of epithelium and basement membrane. There was no evidence of increased ciliary loss or epithelial ulceration after culture.

3.2. Semi-nested RT-PCR

Evidence of RV-16 infection of turbinate mucosae and subsequent viral replication and release into the mucosal surface was assayed by semi-nested RT-PCR. There was no detectable PCR product at 292 bp in the negative control using distilled water, and in the mucosal surface fluid of uninfected control tissue. In this experiment, RVs were applied at the mucosal surface for 4 h. Positive band of 292 bp representing unattached RVs was observed from the mucosal surface fluid taken after 4 h incubation. To remove the unattached RVs from the mucosal surface, four times of extensive washing with PBS was conducted. There was no positive band at the fourth wash fluid indicating that the washing process completely removed the remaining and unattached RVs. The basolateral medium and the PBS used to wash the mucosal surface 48 h later were also assayed by RT-PCR. There was no evidence of the 292 bp band in the basolateral medium indicating that RVs did not contaminate the culture medium. Five of the 10 mucosal surface wash fluid samples of 48 h later revealed a 292 bp RV-specific band, indicating that these cultures had been successfully infected with RV and that these cells subsequently released RVs onto the mucosal surface (Fig. 2).

3.3. ISH

ISH showed no evidence of RV infection of control mucosae, but 6 of the 10 infected turbinate mucosae were virus positive by this assay. In each of the ISH positive mucosae, however, only very low numbers of epithelial cells were ISH positive. These cells were focally located, interspersed by long stretches of noninfected epithelium (Fig. 3). There was no positive signal in the subepithelial lamina propria layer.

3.4. IL-6 and IL-8 quantification

Beginning 24 h after infection, the concentrations of IL-6 and IL-8 in the culture media of infected mucosae were significantly greater than in the media of control mucosae ($p < 0.05$ for each by Wicoxon signed rank test) (Fig. 4).

4. Discussion

Although organ culture of nasal mucosa has been used in various research areas (Schierhorn et al., 1999; Tsang et al., 1994; Jackson et al., 1996), there have been only a

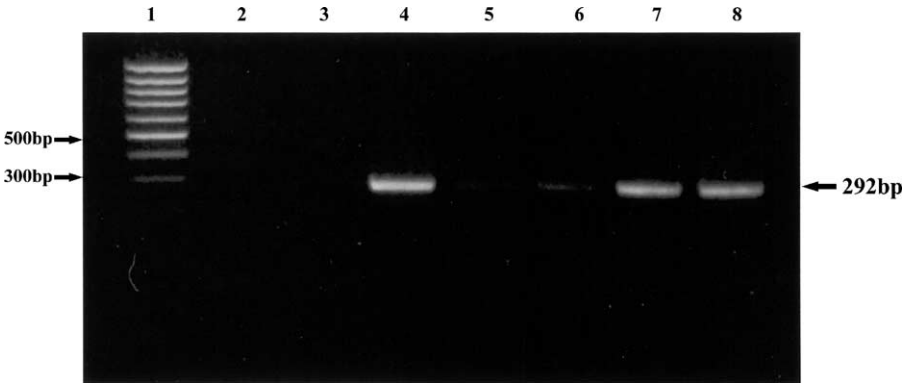

Fig. 2. Semi-nested RT-PCR assay of rhinovirus-16. Lane 1: molecular weight marker; lane 2: negative control for PCR using distilled water; lane 3: supernatant of uninfected control tissue; lane 4, surface liquid 4 h after infection; lane 5: fluid of fourth washing; lane 6: basolateral medium 48 hr after infection; Lane 7, surface fluid 48 hr after infection; Lane 8, positive control using rhinovirus-16. Positive bands of 292 bp are detected in lanes 4, 7, and 8.

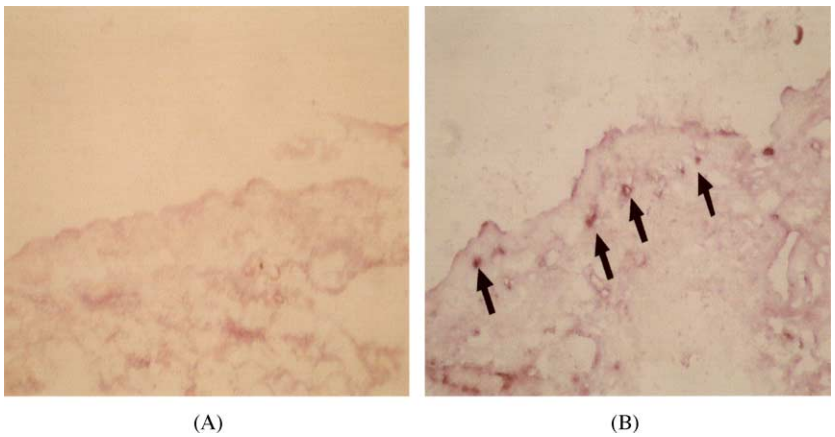

Fig. 3. In situ hybridization using oligonucleotide to detect rhinovirus RNA. (A) Control mucosa, (B) turbinate mucosa infected with rhinovirus-16. While the control mucosa (A) shows no positive spots, infected mucosa shows scattered positive signals (arrows) (original magnification ×200).

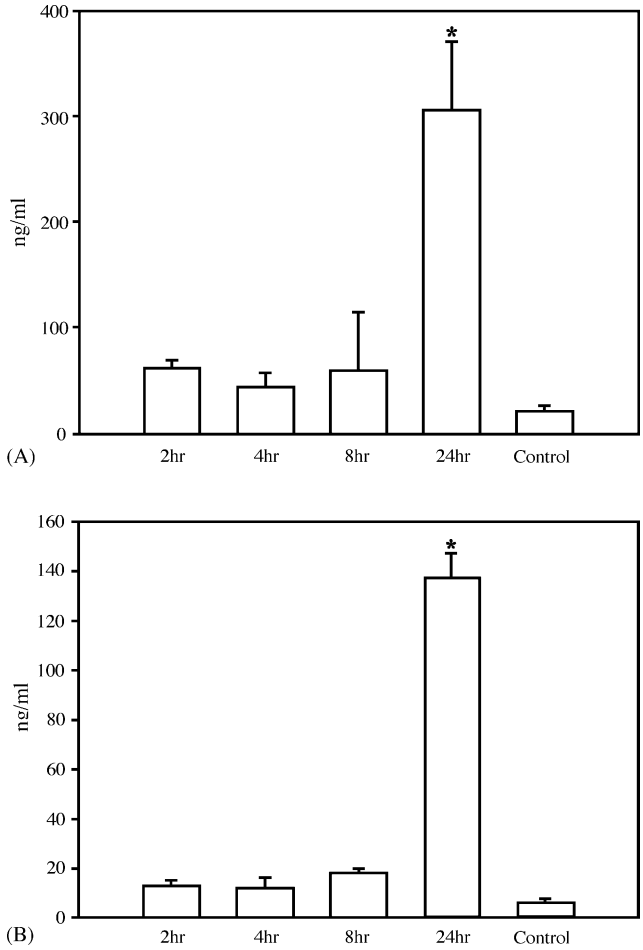

Fig. 4. IL-6 and IL-8 secretion into culture medium after rhinovirus infection. Infected tissue shows significantly increased secretion of IL-6 (A) and IL-8 (B) at 24 h after infection, when compared with control mucosa cultured for 24 h without exposure to rhinovirus. Each bar represents the means ± standard error of five samples. (*$p < 0.05$, Wilcoxon signed rank test.)

limited number of reports in which organ culture was used to study RV infection. For example, trachea has been cultured in screw-capped tubes incubated in a roller drum (Hamory et al., 1977), and fragments of epithelium with adherent submucosa have been cultured from nasal polyps, adenoids, and nasal turbinate in plastic dishes (Winther et al., 1990). The methods used in the previous reports differ from that shown here in that they did use trachea and embryonic tissues, and not use the air–liquid interface method (Hamory et al., 1977; Winther et al., 1990; Higgins et al., 1969; Merigan et al., 1973). Organs can be cultured by submerging the tissue into the media or by the air–liquid interface method, which better mimics physiologic conditions. The air–liquid interface method can also vary according to the matrix, filter paper or gelfoam, by which culture media is supplied. In the present stud, a gelfoam sponge was used to nourish the basolateral side of the tissue and lift up the tissue surface into the air (Schierhorn et al., 1999). When the culture media is not changed, the number of cilia, the ciliary beat frequency, and the mitochondria and nuclei remain the same, and regular change of culture media leaves the cells unchanged for about 20 days (Jackson et al., 1996). In the present study, it was found that the mucosa could be maintained intact for 1 week without any significant epithelial or ciliary damage. Furthermore, since the time in culture never exceeded 4 days, the culture technique we have described should be adequate for future studies.

To demonstrate successful viral infection and replication in epithelial cells, semi-nested RT-PCR on the surface wash fluid 48 h after infection was conducted. Diagnosis of RV infection had traditionally been made by isolating the virus from appropriate specimens after culture. In practice, however, RV culture requires several days and serologic testing is impractical because of the numerous RV serotypes (Greenberg, 2003). In contrast, RT-PCR assays provide a sensitive and specific method for detecting RV (Greenberg, 2003; Pitkaranta et al., 1997). When epithelial cells are infected with RV, only a small fraction of cells are infected. In these cells, viral replication takes place, and RVs are released to

the cell surface, allowing their detection in the surface fluid of epithelial cells. The ability to detect RV infection using semi-nested RT-PCR in the present study differs from conventional methods of viral identification by viral culture in the fibroblasts. Although viral culture and titration is quantitative for RVs, whereas RT-PCR is not, the latter is more rapid (3 days versus more than 1 week). In addition, cell culture methods, while useful in determining the amount of infectious virus, cannot be used to detect very low levels of infectious virus (Myatt et al., 2003). Semi-nested RT-PCR, however, is a highly sensitive method for the detection of RV (Myatt et al., 2003).

In performing RT-PCR, it is essential to completely wash off unattached RVs from epithelial cells after 4 h of adsorption. If RVs are not washed off completely, RT-PCR performed after 48 h in culture can give false positive results. The authors confirmed that extensive washing of the cells completely remove RV by conducting semi-nested RT-PCR on the wash fluid. This study also found that only 5 out of 10 tissue samples showed positive RT-PCR results suggestive of successful infection. This failure to replicate in a particular mucosal tissue samples may have been due to the characteristics of RV-16 strain, to prior immunity of the donors of the turbinate, or to a problem in our methodology. During in vivo RV challenge of volunteers, however, not every volunteer is actually infected (Bruce et al., 1990), for example, one study showed documented RV infection in 75% of the nonallergic subjects (Yuta et al., 1998). Thus, our finding that only 50% of infected samples were positive by RT-PCR may reflect in vivo conditions. In the present study, in order to exclude the effect of mucosal inflammation in the evaluation of the feasibility of the organ culture model, subjects with allergy, paranasal sinusitis, and VRI were excluded from the enrollment. Thus it remains to be determined by future investigation how allergy or sinusitis affect authors' turbinate culture model for RV study. There is one other concern that should be mentioned for turbinate organ culture model. Recently, RV RNA was identified by ISH in 45% of adenoid tissues of the children subjected to adenoidectomy (Rihkanen et al., 2004). Accordingly, there is a chance for a preexisting positivity of RV in the turbinate tissue also. In the present study however, RVs were not detected in the uninfected control tissues, thus the preexisting viral infection could be ruled out. No data are available so far on the detection rate of RV on the uninfected human turbinate tissues. However, further studies are required for a proper evaluation of the feasibility of this study model in relation to the preexisting positivity of RV in the turbinate mucosae.

In the present study, ISH experiments were performed to confirm that viral particles were harbored by epithelial cells. Using an RV-16 probe, RV RNA could be found inside the epithelial cells of 60% of the samples. Five of these six turbinate were also positive by RT-PCR. Finding of RV RNA by ISH but not by RT-PCR in one mucosa is in line with a previous report, showing that ISH was slightly more sensitive than RT-PCR in detecting RV infection of the upper respiratory tract (Pitkaranta et al., 2003). In the samples positive by ISH, however, only a small fraction of epithelial cells were positive. These cells were focally located, interspersed by long stretches of apparently noninfected epithelium. This finding, that RV infection in the nasal epithelium is focal and involves a very small number of cells, is in agreement with evidence from in vivo studies using virus isolation and from ISH studies on nasal polyp fragments infected ex vivo (Bruce et al., 1990; Arruda et al., 1995). Because RV does not cause a marked destruction of the epithelial lining, infection has been considered limited to certain areas in the nasal epithelium (Pitkaranta et al., 2003).

In the authors' present study, using the organ culture model, an increased secretion of IL-6 and IL-8, beginning 24 h after RV infection was demonstrated. The amount of IL-6 and IL-8 secreted has been used as an important parameter of RV-induced pathology (Yuta et al., 1998). IL-8 is of particular interest because its levels increase rapidly and it parallels the time course of nasal symptoms (Gern and Busse, 1999). Thus, finding of the increased secretion of IL-6 and IL-8 suggests that this organ culture model is suitable for studying the outcomes of RV infection. The 24 h time lag is in agreement with an in vivo study showing that cytokine secretion is increased from the second day of infection (Yuta et al., 1998).

In conclusion, the authors have described the use of an air–liquid interface organ culture method for human nasal epithelia. This method closely resembles native epithelium, the primary target of RV infection. Using this model, the authors showed infection with RV and cytokine response similar to in vivo tissues. These findings suggest that the organ culture model used in this study is suitable for studying RV pathogenesis in human respiratory epithelium.

References

Arruda, E., Boyle, T.R., Winther, B., Pevear, D.C., Gwaltney Jr., J.M., Hayden, F.G., 1995. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. J. Infect. Dis. 171, 1329–1333.

Bruce, C., Chadwick, P., al-Nakib, W., 1990. Detection of rhinovirus RNA in nasal epithelial cells by in situ hybridization. J. Virol. Meth. 30, 115–125.

Casasnovas, J.M., Springer, T.A., 1994. Pathway of rhinovirus disruption by soluble intercellular adhesion molecule 1 (ICAM-1): an intermediate in which ICAM-1 is bound and RNA is released. J. Virol. 68, 5882–5889.

Gern, J.E., Busse, W.W., 1999. Association of rhinovirus infections with asthma. Clin. Microbiol. Rev. 12, 9–18.

Gwaltney Jr., J.M., Hendley, J.O., Simon, G., Jordan, W.S.J., 1996. Rhinovirus infections in an industrial population. I. The occurrence of illness. N. Eng. J. Med. 275, 1261–1268.

Greenberg, S.B., 2003. Respiratory consequences of rhinovirus infection. Arch. Intern. Med. 163, 278–284.

Hamory, B.H., Hendley, J.O., Gwaltney Jr., J.M., 1977. Rhinovirus growth in nasal polyp organ culture. Proc. Soc. Exp. Biol. Med. 155, 577–582.

Higgins, P.G., Ellis, E.M., Wooley, D.A., 1969. A comparative study of standard methods and organ culture for the isolation of respiratory viruses. J. Med. Microbiol 2, 109–115.

Jackson, A.D., Rayner, C.F.J., Dewar, A., Cole, P.J., Wilson, R., 1996. A human respiratory tissue organ culture incorporating an air interface. Am. J. Respir. Crit. Care. Med. 153, 1130–1135.

Merigan, T.C., Reed, S.E., Hall, T.S., Tyrrell, D.A., 1973. Inhibition of respiratory virus infection by locally applied interferon. Lancet 17, 563–567.

Myatt, T.A., Johnston, S.L., Rudnick, S., Milton, D.K., 2003. Airborne rhinovirus detection and effect of ultraviolet irradiation on detection by semi-nested RT-PCR assay. BMC Public Health 3, 1–7.

Pitkaranta, A., Arruda, E., Malmberg, H., Hayden, F.G., 1997. Detection of rhinovirus in sinus brushings of patients with acute community acquired sinusitis by reverse transcription-PCR. J. Clin. Microbiol. 35, 1791–1793.

Pitkaranta, A., Starck, M., Savolainen, S., Poyry, T., Suomalainen, I., Hyypia, T., Carpen, O., 2001. Rhinovirus RNA in the maxillary sinus epithelium of adult patients with acute sinusitis. Clin. Infect. Dis. 33, 909–911.

Pitkaranta, A., Puhakka, T., Makela, M.J., Ruuskanen, O., Carpen, O., Vaheri, A., 2003. Detection of rhinovirus RNA in middle turbinate of patients with common colds by in situ hybridization. J. Med. Virol. 70, 319–323.

Rihkanen, H., Carpen, O., Roivainen, M., Vaheri, A., Pitkaranta, A., 2004. Rhinovirus in adenoid tissue. Int. J. Ped. Otorhinolaryngol. 68, 903–908.

Schierhorn, K., Zhang, M., Matthias, C., Kunkel, G., 1999. Influence of ozone and nitrogen dioxide on histamine and interleukin in a human nasal mucosa culture system. Am. J. Respir. Cell Mol. Biol. 20, 1013–1019.

Sethi, S.K., Bianco, A., Allen, J.T., Knight, R.A., Spiteri, M.A., 1997. Interferon-gamma (IFN-gamma) down regulates the rhinovirus- induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. Clin. Exp. Immunol. 110, 362–369.

Terajima, M., Yamaya, M., Sekizawa, K., Okinaga, S., Suzuki, T., Yamada, N., Nakayama, K., Oshima, T., Numazaki, Y., Sasaki, H., 1997. Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1β. Am. J. Physiol. 273, L749–L759.

Tsang, K.W., Rutman, A., Tanaka, E., Lund, V., Dewar, A., Cole, P.J., Wilson, R., 1994. Interaction of *Pseudomonas aeruginosa* with human respiratory mucosa in vitro. Eur. Respir. J. 7, 1746–1753.

van Cauwenberge, P., Ingels, K., 1996. Effects of viral and bacterial infection on nasal and sinus mucosa. Acta Otolaryngol. 116, 316–321.

Winther, B., Gwaltney, J.M., Hendley, J.O., 1990. Respiratoty virus infection of monolayer cultures of human nasal epithelial cells. Am. Rev. Respir. Dis. 141, 839–845.

Yuta, A., Doyle, W.J., Gaumond, E., Ali, M., Tamarkin, L., Baraniuk, J.N., Van Deusen, M., Cohen, S., Skoner, D.P., 1998. Rhinovirus infection induces mucus hypersecretion. Am. J. Physiol. 274, L1017–L1023.

Zhu, Z., Tang, W., Gwaltney Jr., J.M., Wu, Y., Elias, J.A., 1997. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappa B. Am. J. Physiol. 273, L814–L824.