Rhinovirus (RV) infections can alter lower airway physiology and inflammation, yet the characteristics of RV replication in lower airway cells are incompletely understood. An RV serotype 16 (RV16)–specific monoclonal antibody was identified. Immunohistochemistry and an infectious center assay were used to quantitate the infectivity of RV16 in primary bronchial and adenoidal epithelial cells. The proportion of infectible epithelial cells increased with the inoculum but did not exceed 10%. Analysis of bronchial tissue samples infected ex vivo demonstrated a small subset of RV-infected cells in the epithelial layer. These data confirm previous reports that RV infects only a small subset of epithelial cells in upper airway tissues and indicate that lower airway epithelial cells have a similar susceptibility to RV infection. In confirming that RV can infect cells in the lower airway, these results suggest that lower airway dysfunction occurs through this mechanism in susceptible persons.

Human rhinovirus (RV) infections are characteristic upper airway infections, yet they provoke clinically significant lower airway symptoms for patients with asthma, cystic fibrosis, or chronic obstructive pulmonary disease [1–4]. In addition, young children and the elderly may experience lower airway symptoms with RV infections [5]. Other studies have demonstrated that inoculation of the upper airway with RV can cause cellular inflammatory responses in bronchial mucosa, bronchial lavage fluid, and samples of sputum during acute colds [6–8]. Together, the epidemiologic association between RV infections and disturbances in lower airway physiology and the evidence of lower airway inflammatory changes raise the possibility that RV can infect lower airway tissues to promote airway dysfunction.

In vitro studies of RV infection of lower airway cells have shown that RV can replicate in epithelial cells derived from the lower airways and that this process induces secretion of cytokines and chemokines [9, 10]. Furthermore, RV can replicate in lower airway cells, even at core temperature, although greater viral yields from most RV serotypes are obtained at cooler temperatures [9, 11]. In addition, direct mapping of temperatures in the lower airways has demonstrated that, during resting breathing at room temperature, temperatures of the large airways of the lung are 33°C–35°C [12]. These findings suggest that conditions in the lower airway may be favorable for RV replication.

To test the hypothesis that RV infection can extend into the lung, lower airway cells and secretions from experimentally infected volunteers have been analyzed for the presence of virus. Although it has been difficult to culture RV from lower airway secretions [13], RV RNA has been detected in lower airway secretions and cells by reverse-transcriptase polymerase chain reaction and by in situ hybridization [14, 15]. Together, these findings establish that RV can grow in the lower airway, although the quantity of RV replication in the lower airway and the specific cells that support RV replication are unknown.

Patterns of RV growth in epithelial cells from the upper airway have been defined. Examination of mucosal biopsy specimens obtained during RV infection reveals little or no evidence of cytopathic effects on the epithelium [16, 17]. Analysis of the pattern of infection by point cultures of the airway indicates that RV produces a spotty infection of the upper airway with small foci of infected cells [18], and this has been defined at the cellular level by examination of nasal mucosal biopsy specimens with in situ hybridization [19, 20]. In these studies, small clusters of intensely infected cells were observed in the epithelial layer of the upper airway.

Collectively, this information indicates that RV replication is limited to a small number of epithelial cells in the upper airway. However, the extent to which RV replicates in the lower airway in vivo and the pattern of infection in lower airway cells have not been determined. To begin to address these outstanding questions, we have developed methods, including immunohis-
tochemical analysis of infected tissue by use of a monoclonal antibody (MAb) to RV16, to define patterns of RV infection in primary cultures of lower airway bronchial epithelial (BE) cells. The objective of this study was to use these techniques to identify which cells can be infected and to compare the frequency of cells susceptible to RV infection in upper versus lower airway epithelium.

Materials and Methods

Cells, virus, and antibodies. H-HeLa cells [21] were grown in Eagle MEM (EMEM; Life Technologies) supplemented with non-essential amino acids, t-glutamine, and antibiotics (EMEMsupp). This medium was further supplemented with 10% donor calf serum with iron (Hyclone).

Primary and low-passage epithelial cells were derived from surgical specimens of normal human bronchi (from lung transplant donors) or adenoids and were isolated by a modification of the method described by Schroth et al. [9] and Wu et al. [22]. All specimens were cultured on collagen-coated plastic tissue culture labware in BE cell growth medium (BEGM; BioWhittaker). For some experiments, adenoids from 2 or 3 patients were pooled.

RV16 was grown and titered in HeLa cells [23]. Mouse hybridomas secreting MAbs against RV16 were produced by HTI Bio-Products, using purified RV16 (provided by Wai-Ming Lee, Institute for Molecular Virology, University of Wisconsin). Antibodies were produced by growing the hybridomas as mouse ascites. One of the antibodies, R16-7, was purified with a protein A HiTrap column (Sigma Chemical). The stock of purified antibody had a protein concentration of 4.5 mg/mL. The specificity of the antibody for human RVs was verified by Western blot (figure 1). This antibody reacts with both RV16 and the closely related RV1A.

Immunoblotting. Protein electrophoresis was performed on 12% SDS-PAGE gels, using extracts of uninfected HeLa cells and cells infected with 5 different RV serotypes. Samples were transferred onto polyvinylidene fluoride membranes. The membranes were treated with blocking buffer and were incubated for 60 min with a 1:2000 dilution of antibody R16-7 in blocking buffer. The membranes were rinsed 3 times in wash buffer and then were incubated with horseradish peroxidase anti-mouse antibodies in blocking buffer. After a wash, bands were visualized by chemiluminescence with the ECL Western blotting detection reagent (Amersham).

Low-dose viral yield studies. Monolayers of HeLa or primary BE or adenoidal epithelial (AE) cells in 24-well plates were washed once with Dulbecco’s PBS, and triplicate wells were infected with 50 μL of suitably diluted RV16. Virus was diluted into PBS containing 0.1% bovine serum albumin (BSA). Virus was allowed to absorb for 1 h at room temperature. Unabsorbed virus was removed by washing. Nutrient medium (EMEMsupp without serum for HeLa cells and BEGM for epithelial cells), 0.5 mL/well, was added, and the cells were incubated at 34°C for 2, 24, 48, or 72 h. Cells were lysed by 3 cycles of freezing and thawing, and the total virus in each sample was determined by TCID50 assay [24]. Four tubes were incubated for each 10-fold dilution. When no virus was detected, the result was recorded as log10 TCID50/mL = 0.4 (equals half the limit of detection). Since progeny virus does not appear for ≥4 h after infection, the remaining virus infectivity after 2 h of incubation at 34°C was taken as a measure of input virus that had not “eclipsed,” or lost infectivity after attachment to cells.

Infectious center assays. HeLa cells or primary airway cells were infected, as described above. After 3 h of incubation at 34°C, the cells were trypsinized, counted, diluted if necessary, and plated over confluent monolayers of HeLa cells in 6-well tissue culture plates. Cells were allowed to settle during a further 3.5 h of incubation at 34°C, and then the liquid medium was carefully removed and was replaced with 1.5 mL of EMEMsupp containing 0.04 M magnesium chloride, 0.1% BSA, and 0.8% noble agar. When this layer had solidified, an additional 1.5 mL of the above medium, lacking agar, was added. The plates were incubated at 34°C for 2 days. Then the cells were fixed by the addition of 3.4% formaldehyde to the medium and were stained with 0.1% crystal violet in 20% ethanol. Plaques were counted to enumerate infected cells.

Immunocytochemistry. We used immunocytochemistry to evaluate monolayers of HeLa and airway epithelial cells and pieces of bronchial and adenoidal tissue that were infected ex vivo. For evaluation of cell monolayers, primary airway cells or HeLa cells were grown on collagen-coated glass chamber slides; infected with RV16, as detailed above; and incubated at 34°C for 6.5 or 20 h. Cells were washed 3 times with PBS to remove extracellular virus, and the plastic chambers were removed. Cells were fixed in acetone, permeabilized with saponin (Sigma), and stained with MAB R16-7 at a 1:30,000 dilution, with normal mouse immunoglobulin (Zymed Laboratories) or with MAbs specific for intercellular adhesion molecule 1 (ICAM-1, clone 15.2; Ancell). We used the alkaline phosphatase–anti–alkaline phosphatase method [25]. Cells were counterstained with hematoxylin. To obtain counts of stained and unstained cells, photographs were taken from stained slides in a systematic way, to eliminate bias. A randomly selected area was photographed, and then the slide was moved one field to the right and photographed again. When the edge of the slide was reached,
the slide was moved one field down, and subsequent photographs were taken to the left. Stained and unstained cells were counted by hand, using a manostat colony counter.

For in situ examination of bronchi infected ex vivo, small pieces of fresh tissue were washed to remove red blood cells, cut into pieces ~1–2 mm in diameter, and incubated at 34°C in BEGM with virus. After 20 h of incubation, they were washed 3 times to remove free virus, fixed, and paraffin embedded. Sections (4 μm) were cut, the paraffin was removed, sections were rehydrated, and tissues were stained, using MAb R16-7 and the EnVision® system (horseradish peroxidase–diaminobenzidine; Dako).

Pieces of adenoidal tissue were infected by addition of virus directly to the surgical specimen in Ham’s F12/Dulbecco’s MEM, plus penicillin and streptomycin. They were gently agitated for 6 h at 35°C and then were processed as for bronchial tissue.

**Flow cytometry.** Monolayers of BE cells were detached by a 40-min incubation at room temperature in PBS containing 10 mM EDTA and 25 mM HEPES. The cells were then collected and were diluted in an equal amount of PBS containing 0.2% BSA. After centrifugation, the cells were resuspended in flow cytometry (FACS) buffer (Becton Dickinson; PBS containing 2% BSA and 0.2% sodium azide) at 1.5–3 × 10⁵ cells/mL. In total, 100 μL of cells was added to 100 μL of FACS buffer plus 5 μL of phycoerythrin-labeled anti–ICAM-1 MAb (clone LB-2; Becton Dickinson) or 5 μL of an isotype control mouse IgG (Becton Dickinson). The cells were incubated for 30 min on ice with shaking and then were centrifuged for 10 min at 400 g at 4°C. The cell pellet was resuspended in 0.5 mL of FACS buffer containing 2 mg/mL propidium iodide to identify live cells.

HeLa cells were pelleted, resuspended in PBS plus 1% calf serum, and then incubated on ice for 30 min with 5 μL of phycoerythrin-labeled anti–ICAM-1 MAb (clone LB-2; Becton Dickinson) or 5 μL of an isotype control mouse IgG (Becton Dickinson). The cells were washed in 10 mL cold PBS plus 10% calf serum and were resuspended in 0.5 mL of the same buffer.

For each cell sample, 10,000 events were collected by FACSScan (Becton Dickinson), and data were analyzed by CellQuest software (Becton Dickinson), with fluorescence expressed as fluorescence units (FU). To measure the expression of ICAM-1, we gated on live cells stained with the isotype control antibody and measured the percentage of cells stained with the anti–ICAM-1 antibody that exceeded that gate.

**Statistical analysis.** Titers of infectious virus were expressed in log₁₀ TCID₅₀ units. The effect of cell type on viral titers was evaluated with 2-way analysis of variance. Factors evaluated were cell type and the initial inoculum of RV16. Significant differences were then assessed by Student’s t test with Bonferroni corrections. P < .05 was considered to be significant. We used SigmaStat software (Sigma) for data analyses.
Results

Low-dose inoculation of epithelial cells. To determine the minimum amount of virus needed to infect cultures of BE and AE cells, monolayers of primary cells were infected with diluted RV16 (50 \( \mu \)L/well), and quantitative viral cultures were performed on samples harvested 2, 24, 48, and 72 h after the end of the virus absorption period (figure 2A and 2B). For comparison, the same experiment was performed with HeLa cells (figure 2C). In these experiments, there were \( \sim 2-5 \times 10^5 \) cells/well, so that all virus inputs were \( <1 \) infectious dose/cell. The results showed that far less virus was needed to initiate productive infection in HeLa cells than in BE or AE cells. With HeLa cells, 10 TCID\(_{50}\)/well invariably led to vigorous virus production; only when the average input dose was reduced to 1 TCID\(_{50}\)/well did some wells fail to produce virus. In BE or AE cells, \( \sim 1000 \) TCID\(_{50}\)/well was required to establish a productive infection.

In HeLa cultures, the virus clearly spread from cell to cell, and the viral yield continued to mount over 3 days. In BE cells, there was rarely an increase in titer between 24 and 48 h, and by 72 h, the measurable titer had dropped, perhaps due to release of viral inhibitory factors into the medium. Maximum yields from BE cells were \( \sim 10^4 \) TCID\(_{50}\)/mL, compared with maximum yields of \( 10^7 \) TCID\(_{50}\)/100 \( \mu \)L for HeLa cells. Maximal yields from AE cells were slightly, but not significantly, higher than those from BE cells. Even after 72 h, there was no observable cytopathic effect in the BE or AE cell monolayers. In contrast, HeLa cells showed extensive cytopathic effect whenever the virus yield reached \( \geq 10^5 \) TCID\(_{50}\)/mL.

Because these experiments were performed with RV16 that had been passaged multiple times in HeLa cells, we performed parallel experiments in AE cells, comparing the HeLa-adapted RV16 with a stock of RV16 only 3 passages after isolation from a human, a stock that grows poorly in HeLa cells. Table 1 shows that the low-passage virus did not produce more virus in primary airway epithelial cells than the HeLa-adapted virus.

**Cell-related differences in viral eclipsing.** In addition to the cell-related differences in RV replication, another difference between primary cells and HeLa cells was the amount of infectious virus associated with the cells 2 h after inoculation. This measurement was included to estimate the efficiency of the uncoating, or eclipse, process. The binding of picornaviruses to their receptors is a necessary first step in the conformational changes (“eclipse”) that lead to release of viral RNA into the cell, and these viruses lose infectivity during this process. At 2 h after infection, new progeny viruses have not yet been synthesized, so infectivity in these samples represents virus that has bound but
Figure 5. Immunohistochemistry of infected or uninfected cells stained with monoclonal antibody R16-7 (panels A–E) or with mouse isotype-control antibody (panel F). Cells were counterstained with hematoxylin. Bar, 50 µm. A, Bronchial epithelial cells infected 6 h before fixation; B, uninfected bronchial epithelial cells; C, adenoidal epithelial cells infected 30 h before fixation, with infected cells showing typical cytopathic effect; D, uninfected adenoidal epithelial cells; and E and F, HeLa cells infected 6.5 h before fixation. Many infected cells show typical cytopathic effect.
not eclipsed. In our experiments, more infectious virus was associated with BE cells or AE cells than with HeLa cells (figure 3), suggesting a less efficient eclipse mechanism in the airway cells.

**Estimation of the frequency of infectible cells.** The maximal yields of RV from the airway cells were 3–4 orders of magnitude less than those from HeLa cells. This could reflect markedly lower yields per cell and/or the inability to infect most of the airway cells in cultures. To resolve this issue, and to compare the frequency of infectible cells in the upper versus lower airway, the numbers of infectible cells in AE and BE cell cultures were estimated, using 2 different techniques. First, an infectious center assay was performed in which cell monolayers were infected and then trypsinized, counted, and seeded onto confluent monolayers of HeLa cells, which acted as indicator cells. When an infected cell settles onto the HeLa monolayer, a plaque of infected HeLa cells should develop, and so the number of plaques is proportional to the number of infected cells in the test sample.

Analysis of BE (figure 4A), AE (figure 4B), and HeLa cells (figure 4A and 4B) by this technique demonstrated that, although a 10-fold increase in input virus led to a 10-fold increase in the percentage of cells infected for all cell types, until maximal values were reached, more HeLa cells were infected at each RV infecting dose. For example, all the HeLa cells were infected with an input of ~10 TCID_{50} per cell. For the BE or AE cells, at most 5%–10% of the cells were infected, even with very large (500 TCID_{50}/cell) inocula. There was also considerable variability between BE or AE cell cultures from different donors.

Immunohistochemical staining with the R16-7 antibody was used as a second method of measuring the number of RV-infectible cells. HeLa and primary airway epithelial cells were grown on collagen-coated glass slides, infected with 15–150 TCID_{50}/cell of RV16, and stained with antibody R16-7. Primary airway cells (figure 5A and 5C) show occasional cells that stained for the presence of RV16. For these pictures, areas of the slides were chosen that were relatively heavily infected. In the HeLa cell culture (figure 5E), the majority of cells stained positively. Many of these cells were beginning to round up and detach from the glass surface. The intensity of staining varied in cells of all origins. Uninfected BE cells (figure 5B) and AE cells (figure 5D) showed no evidence of intracellular staining with R16-7. Neither HeLa cells (figure 5F) nor BE cells (data not shown) stained with an isotype control MAb.

Quantitative analysis of airway epithelial cells stained with antibody R16-7 showed that contagion rates did not exceed 6%. HeLa cells infected at ~17 TCID_{50}/cell were ~97% positive for the presence of virus. These results agree well with the results from infectious center assays (table 2).

**In situ analysis of RV infection.** To determine the pattern of infection of cells in pieces of tissue infected ex vivo, small pieces of bronchial tissue were incubated with RV16 (10^7–10^8 TCID_{50}/mL) for 20 h; after extensive washing to remove extracellular virus, the tissue was paraffin embedded, sectioned, and stained for the presence of RV16. A small subset of epithelial cells had cytoplasmic staining for RV16 (figure 6A). In some cases, several adjacent cells that had detached from the tissue surface stained positively for the presence of virus. In some sections (data not shown), a rare subepithelial cell stained positively with this RV16 antibody. Intracellular staining was not observed in uninfected tissues stained with R16-7 (data not shown) or in infected tissue stained with a mouse isotype control antibody (figure 6B).

Pieces of adenoidal tissue were similarly infected but only for 6 h, to observe the first round of viral replication. Cells were extensively washed to remove unabsorbed virus and were processed for immunohistochemistry by paraffin embedding, sectioning, and staining with the R16-7 antibody (figure 6C). At this early stage of infection, only a few ciliated epithelial cells were clearly infected, with extensive staining of the cytoplasm. No infected cells were observed in subepithelial layers. Several ciliated cells were stained for the presence of virus on their exposed surfaces. This virus may have adsorbed to the cells but may not have internalized or set up a productive infection. This adsorption may or may not indicate specific binding to the ICAM-1 receptor.

**Flow cytometry analysis of primary BE and HeLa cells for the presence of ICAM-1.** One possible explanation for the difficulty of infecting primary airway epithelial cells might be a paucity of ICAM-1 molecules constitutively expressed on the surface of these cells, since ICAM-1 is the receptor that RV16 and most other human RVs use to bind to cells. Specific binding is necessary to initiate the uncoating process, and it is possible that a threshold number of ICAM-1 molecules must be bound for uncoating to take place. We investigated the amount of ICAM-1 on BE cells, using flow cytometry (figure 7A). The curve for cells stained with the ICAM-1 antibody was only slightly broader and shifted to the right (median FU, 8.1 vs. 6.5), compared with that for cells stained with the isotype control, suggesting that most cultured BE cells express only small amounts of ICAM-1 on their surface. In addition, ~2% of epithelial cells

<table>
<thead>
<tr>
<th>Cell origin</th>
<th>MOI, pfu/cell</th>
<th>Cells counted</th>
<th>Infected cells, no. (%)</th>
<th>MOI, pfu/cell</th>
<th>% Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoid</td>
<td>35</td>
<td>10,300</td>
<td>231 (2.2)</td>
<td>36</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>17,411</td>
<td>129 (0.74)</td>
<td>29</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>360</td>
<td>5.8</td>
</tr>
<tr>
<td>Bronchus</td>
<td>30</td>
<td>8,192</td>
<td>80 (0.98)</td>
<td>41</td>
<td>0.34*</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>520</td>
<td>5.9</td>
</tr>
<tr>
<td>HeLa</td>
<td>16.7</td>
<td>634</td>
<td>613 (97)</td>
<td>13.9</td>
<td>99</td>
</tr>
</tbody>
</table>

NOTE. ND, experiment not done.

*For this experiment, infectious center assay and immunohistochemistry were done with cells seeded from same surgical sample.
had ICAM-1 expression that was clearly outside the gate for cells stained with the isotype control antibody (data not shown). In agreement with these findings, BE cells cultured on collagen-coated slides and stained for the presence of ICAM-1 showed only a few brightly stained cells (figure 7B). By comparison, HeLa cells expressed greater amounts of ICAM-1, as indicated by flow cytometry (figure 7C).

Discussion

In addition to confirming previous reports that lower airway cells can be infected with RV [9, 10], our results indicate that primary AE and BE cells are similarly susceptible to RV infection in vitro. Accordingly, we found that only a small subset of either upper or lower airway cells could be infected with RV. With large inoculating doses of virus (100–500 TCID₅₀/cell), ≤10% of the cells in primary airway epithelium cultures were infected. It is likely that viral concentrations during natural infections are considerably less and that even fewer cells are infected and ultimately lysed by RV. This is consistent with the fact that little or no mucosal damage has been observed in biopsy specimens of airway epithelium from infected individuals [26].

These experiments also demonstrated large differences in the degree of viral replication between HeLa cells and the nontransformed airway epithelial cells. For example, at an input of 10⁴ TCID₅₀/well, HeLa cells produced 10⁶.2 TCID₅₀/mL over 24 h,

![Figure 6](image_url)

Figure 6. Immunohistochemistry of airway tissue infected ex vivo. Bronchial tissue pieces were inoculated ex vivo and were incubated in tissue culture medium for 24 h (A). After washing to eliminate extracellular virus, the tissue was embedded in paraffin, sectioned, and stained for the presence of rhinovirus serotype 16 (RV16), using the EnVision® system (Dako). Cells were counterstained with hematoxylin. Uninfected bronchial tissue piece (B) was processed as in panel A. Adenoidal tissue piece infected ex vivo for 6 h (C), after washing to eliminate unabsorbed virus, was paraffin embedded, sectioned, and stained for the presence of RV16, using the EnVision® system. Bar, 50 μm.
whereas BE cells produced only $10^5$ TCID$_{50}$/mL (figure 2). However, we have shown that only ~1% as many BE cells were infected as HeLa cells (figure 4). Thus, the large difference in yield of RV between BE cells and HeLa cells is at least partially due to a difference in the number of cells infected.

Cell-specific differences in the kinetics of viral replication were also noted. When viral yield was measured on subsequent days after infection with very small doses of input virus, the recovered infectivity increased daily by at least an order of magnitude in the HeLa cultures, until the titers were $>10^7$ TCID$_{50}$/mL (figure 2). In contrast, the virus did not appear to spread significantly in the BE cultures. This suggests that the BE cells, unlike the HeLa cells, produced a factor that inhibited viral spread. Alternately, limiting amounts of ICAM-1 on BE cells may have reduced the possibility of viral spread, compared with HeLa cells. These experiments used a HeLa-adapted strain of RV16, and this could have affected the relative growth rates of the virus in HeLa cells versus nontransformed airway epithelial cells. However, similar experiments using low-passage RV16 that was not adapted to HeLa cells produced indistinguishable results (table 1).

Another difference between the airway epithelial and HeLa cells was in the degree to which the cell-bound virus was eclipsed. Eclipse, which represents the initiation of uncoating, is measured as the loss of measurable infectivity in virus exposed to cells at physiological temperatures [27]. We found less eclipse of RV16 in AE and BE cells than in HeLa cells. Although the

**Figure 7.** Intercellular adhesion molecule 1 (ICAM-1) expression on bronchial epithelial cells vs. HeLa cells. Histogram (A) shows flow cytometric data from 10,000 bronchial epithelial cells stained with a phycoerythrin-labeled anti–ICAM-1 monoclonal antibody (MAb) or a mouse IgG isotype control antibody. FU, fluorescent units; no. of cells, cells per 10,000 cells counted. B. Uninfected bronchial epithelial cells grown on a collagen-coated slide, fixed, and stained for the presence of ICAM-1. Bar, 50 μm. Histogram (C) shows flow cytometric data from 10,000 HeLa cells stained with a phycoerythrin-labeled anti–ICAM-1 MAb or with a mouse IgG isotype control antibody.
reason for the slower rate of eclipse is unknown; potential explanations include a low density of cell-surface ICAM-1, differences in the kinetic properties in membrane mobility, or an internal block to uncoating.

Only a small subset of lower airway epithelial cells could be infected with RV16, which is similar to the pattern of infection that has been described in upper airway cells [19, 20]. Our experiments used 1 serotype of RV, but Arruda et al. [20], using RV14 and RV39, found similar results, suggesting that this is a general property of RVs. There may be some variability in the number of cells infected that correlates with the receptor to which the RVs bind. Schroth et al. [9] found that RV49, an RV that binds to the low-density lipoprotein receptor, caused more cytopathogenicity in primary BE cell cultures than did RV16.

The infectible cells, which stained intensely for viral proteins, reside predominantly in the outer epithelial layer, although a few infected cells in the subepithelial layer were also detected. With very high doses of RV, a proportionally greater number of cells were infected, and this provides a potential mechanism to explain clusters of infected cells that have been observed in the upper airway. In this scenario, an initially low inoculum of virus would infect relatively few highly susceptible airway epithelial cells. After viral replication and lysis of the infected cell, cells in the immediate environment could be exposed to large amounts of virus, and so even relatively resistant cells could be infected.

Why are some airway cells infected and others not? One possible reason might be the density of ICAM-1 on the surface of the cells. Our studies with flow cytometry and immunohistochemistry have shown a low basal expression of ICAM-1 (figure 7), although higher ICAM-1 expression could be demonstrated on a small percentage of cells. A similar pattern of staining for ICAM-1 has been observed in human adenoidal tissue, in which a subset of nonciliated epithelial cells were found to have high ICAM-1 expression [28]. In contrast, in this study, most of the epithelial cells that stained positively for RV16 in infected adenoidal tissue were ciliated. Other potential explanations for enhanced susceptibility to RV in a subset of epithelial cells might be a selective deficiency in key antiviral pathways or selective expression of intracellular proteins that are required for RV replication [29, 30].

In summary, these experiments have provided a clearer picture of the susceptibility of lower airway epithelial cells to infection with RV. Our data indicate that BE cells are as susceptible to RV infection as are cells lining the adenoids, where the greatest amount of virus can be recovered from the upper airway during natural colds [18]. During the coughing and sneezing induced by RV colds, there is little doubt that the lower airway is inoculated by droplets containing infectious virus, and it has recently been demonstrated that RV can replicate in lower airway tissues in vivo in experimentally infected volunteers [14, 15]. What remains to be determined, however, is whether the degree of RV replication in the lower airway is clinically significant during naturally acquired colds and, in particular, whether induction of lower airway symptoms and changes in lower airway physiology during RV colds may be a result of enhanced lower airway infection. The development of an RV-specific MAb provides a sensitive tool to further test this hypothesis by evaluating lower airway biopsy specimens during natural or experimentally induced RV infections.

Acknowledgments

We thank Diane Heatley and Scott McMurray for providing the adenoidal tissue, Robert Love for providing the bronchial specimens, and Wai Ming Lee for providing highly purified RV16.

References