Surface Expression of Intercellular Adhesion Molecule 1 on Epithelial Cells in the Human Adenoid

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Human rhinoviruses enter the host by way of the nose and conjunctiva. Intercellular adhesion molecule 1 (ICAM-1) is the cellular receptor for the majority of rhinoviruses. ICAM-1 expression on the luminal surface of epithelial cells in the upper airway may be an important determinant of virus localization in the airway. Eighteen adenoids and 5 nasopharyngeal biopsies were evaluated by immunohistochemistry for surface expression of ICAM-1. Heavy immunoreactivity of ICAM-1 was found on the surface of a small number of single nonciliated cells in the lymphoepithelium. Squamous epithelial cells showed minimal to no staining, and ciliated epithelium had positive ICAM-1 staining of the basal cells but not on the ciliated border. The localization of ICAM-1 expression to specific, limited areas of the surface epithelium of the nasopharynx may have important implications in the pathogenesis of rhinovirus infections, especially initiation of the host response to rhinovirus.

Intercellular adhesion molecule 1 (ICAM-1) serves as the surface receptor on human cells for entrance of the majority of rhinoviruses [1, 2]. Binding of rhinoviruses to the ICAM-1 receptor is therefore the first step in infection of susceptible cells in the upper airway mucosa. The distribution of immunoreactive ICAM-1 (CD54) on the surface of the epithelium covering the nasopharyngeal tonsil (adenoid) may be important in the pathogenesis of rhinovirus infection, since the earliest site of rhinovirus recovery in normal volunteers with experimental rhinovirus infection is from the nasopharynx [3].

Although ICAM-1 is expressed on a variety of human cell types [4], it has not been detected on the normal nasal epithelium [5, 6]. ICAM-1 expression on the surface of epithelium overlying the nasopharyngeal lymph node [7] has not been evaluated. In this study, we used an immunohistochemical staining technique to examine the surface of nasopharyngeal epithelium for ICAM-1 expression.

Materials and Methods

Tissue. Adenoids removed from 18 children (age range, 2–10 years) because of enlarged adenoids or chronic middle ear effusion (or both) were utilized. None of the children had signs of acute infection at time of surgery. After removal, each adenoid was placed in Hanks’ balanced salt solution containing penicillin (100 μg/mL), streptomycin (100 μg/mL), and amphotericin B (1 μg/mL) for transport to the laboratory. The specimens were fixed within 4 hours after removal from patient. Only intact adenoids measuring at least 1.5 × 3 cm² were used. The adenoid was oriented and cut horizontally in the middle, and a 2-mm² piece was cut from the epithelial surface on the wall of a fold/ridge [7].

In addition, biopsies (3 mm³) from the posterior nasopharyngeal wall were obtained from 5 healthy young adults (age range, 18–25 years) with cutting forceps after topical anesthesia with 4% lidocaine.

Techniques for tissue preparation and embedding. Three different techniques were used to prepare tissue for staining for ICAM-1:

Four adenoids and the 5 nasopharyngeal biopsies were snap-frozen in liquid nitrogen at −70°C immediately after removal. Sections (4–6 μm thick) were cut on a cryostat, dried on slides, fixed in cold acetone for 10 min, and hydrated with PBS (pH 7.4) containing 1% fetal calf serum.

Fourteen adenoids were fixed in acetone at −20°C overnight and embedded in glycol-methacrylate resin (Polysciences, Warrington, PA) as described by Casey et al. [8]. Sections (2 μm thick) were cut using disposable steel knives. Cut sections were placed on slides coated with poly-L-lysine (Sigma, St. Louis) and held in place with Tissue Tack (Polysciences). This method preserves the antigens and provides excellent morphology.

Portions of 2 adenoids were fixed in acetone overnight at −20°C and embedded in paraffin. Sections (4 μm thick) were placed on poly-L-lysine–coated slides and dewaxed prior to staining.

Monoclonal antibodies (MAbs) and staining techniques. Two different MAbs to ICAM-1 (CD54) were used: one commercial (Dako, Carpinteria, CA) and the other, designated c78.4, produced by one of us by inoculation of mice with HeLa cells. Culture supernatant from hybridoma c78.4 prevented infection of HeLa cells by human rhinovirus HRV14 and HRV3 but did not block infection with HRV2. Both MAbs were used in a 1:100 dilution.

Endogenous peroxidase activity was blocked with methanol-H₂O₂, and endogenous biotin activity in sections was blocked with avidin-D and biotin solutions (Vector Laboratories, Burlingame,
CA) before incubation with antibodies. Sections were then incubated for 30 min with the primary antibody, which was the mouse MAb to human cell markers. After that, sections were sequentially incubated with secondary antibody (biotin-conjugated horse antibody to mouse) and avidin-horseradish complex (Vectastain ABC ELITE kit; Vector). Sections were washed with PBS between steps. Peroxidase was visualized with 0.01% 3,3-diaminobenzidine (Polysciences) in PBS with 0.01% hydrogen peroxide. Sections were counterstained with Harris’ hematoxylin.

Staining specificity controls. Every staining procedure (run) included a slide with PBS substituted for primary antibody as a negative control. The positive control for CD54 was staining of ICAM-1 on endothelial cells of vessels in the lamina propria. Two additional procedures were carried out on plastic sections to confirm the staining specificity of ICAM-1. First, an irrelevant antibody in a 1:1000 dilution was used instead of primary antibody. The irrelevant antibody was MOPC-21, a mouse myeloma protein (IgG1 isotope) purified from ascites fluid, which has no known antigen. Second, in one run, a purified soluble ICAM (tICAM453, 7.5 mg/L) was incubated with anti–ICAM-1 (1:1000) for 10 min prior to the staining procedure to determine whether soluble ICAM would block the MAb c78.4 staining of endothelial and epithelial cells.

Analysis. All specimens were evaluated by light microscopy and representative photos were obtained.

Results

All 18 adenoid specimens and 5 nasopharyngeal biopsies showed abundant lymphatic tissue in close contact to the mucosal epithelium. The epithelium consisted of ciliated or squamous cells or patches of lymphoepithelium [7, 9, 10].

Frozen and paraffin-embedded sections (4–6 μm thick) showed ICAM-1 expression on the surface and deeper layers of the lymphoepithelium (figure 1A). Ciliated epithelium next to the lymphoepithelium showed minimal to no ICAM-1 staining of the surface of the ciliated cells, while some staining of the basal cell layer was apparent (figure 1A). Squamous epithelium showed minimal to no staining (not shown). Distinct ICAM-1 expression on the surface of individual epithelial cells in the lymphoepithelium (figure 1A) was difficult to determine because of the positive ICAM-1 staining of adjacent intraepithelial lymphocytes.

Plastic-embedded sections (2 μm thick) allowed evaluation of ICAM-1 expression on individual epithelial cells. ICAM-1 was expressed on the luminal surface only on nonciliated epithelial cells in the lymphoepithelium (figure 1B, C); ciliated cells and squamous cells did not express ICAM-1 on their surfaces. ICAM-1 was also consistently present in the microvascular endothelial cells and in the germinal centers in all adenoid specimens (not shown).

Controls. Substitution of PBS or irrelevant antibody for primary antibody resulted in no staining; incubation of tICAM453 with anti–ICAM-1 blocked staining. MAb c78.4 and commercial CD54 showed the same specificity when compared in 3 specimens.

Figure 1. ICAM-1 expression on epithelial surface of nasopharyngeal lymph node. A, Frozen section of nasopharyngeal biopsy specimen with immunoperoxidase staining of ICAM-1 showing lymphoepithelium (LE) at left with shift (arrow) to ciliated epithelium (CE) at right (original magnification, ×100). Lymphoepithelium shows heavy expression of ICAM-1. ICAM-1 is not expressed on luminal surface of ciliated cells, but deeper part of ciliated epithelium showed expression of ICAM-1. B. Plastic-embedded section of adenoid specimen stained with immunoperoxidase for ICAM-1 showing positive staining of single nonciliated cells on epithelial surface facing crypt (original magnification, ×200). C. Higher magnification of portion of plastic-embedded section shown in B (original magnification, ×400).

Discussion

Strong immunoreactivity of ICAM-1 was demonstrated on the luminal surface of individual nonciliated epithelial cells of adenoids from children and nasopharyngeal mucosa from young adults. Ciliated and squamous cells had no detectable ICAM-1 expression on the epithelial surface of the adenoid
mucosa. Immunoreactivity was also seen in microvascular endothelial cells, in the germinal centers, and in the basal layer of the ciliated epithelium.

This distribution of ICAM-1 positivity confirms earlier findings of Dustin et al. [4], who reported ICAM-1 positivity of the vascular endothelium, of dendritic reticular cells, and of B cells in the germinal centers of the pharyngeal tonsils. Dustin et al. did not report ICAM-1 expression on the epithelial cells of the pharyngeal tonsil. Ciprandi and colleagues [5, 6] found no ICAM-1 expression on epithelial cells retrieved by centrifugation from nasal washes in the normal state. However, ICAM-1 staining was present in such cells obtained after topical allergen challenge of the nose, but the type of epithelial cells with ICAM-1 staining was not described. Brandtzaeg [11] also found that the surface epithelium of nasal biopsies from patients with moderate rhinitis was virtually negative for ICAM-1 expression. Human rhinovirus has been detected in sloughed nasal ciliated cells during rhinovirus colds, but we did not detect ICAM-1 expression on the epithelial surface of the ciliated cells [12]. Since rhinoviruses gain entrance to the cells by the ICAM-1 receptor, this discrepancy suggests either that there is a lack of sensitivity of our staining method or that surface ICAM-1 may be up-regulated during rhinovirus infection.

Plastic-embedded sections (2 μm thick) were clearly superior to frozen or paraffin-embedded sections for evaluation of individual epithelial cells in the nasopharyngeal mucosa. Many of the single nonciliated epithelial cells in the lymphoepithelium expressing ICAM-1 on the surface had a “dome” appearance in the plastic sections. These cells constituted 1%–20% of the nonciliated cells in the lymphoepithelium. Human rhinovirus has been shown to replicate in a small number of nonciliated epithelial cells of the adenoid [13], which may be the epithelial cells expressing high ICAM-1 immunoreactivity. M cells have been demonstrated in the nasopharyngeal epithelium [7, 9] by scanning and transmission electron microscopy. M cells are single, nonciliated epithelial cells in lymphoepithelium that have a dome shape [7], similar to the ICAM-1-positive cells visualized in our plastic-embedded specimens. Whether the heavy ICAM-1 immunoreactivity on single nonciliated cells in the adenoid is part of the normal steady state or is a response to up-regulation is not known.

The transepithelial pathway for antigens to cross the nasopharyngeal mucosa is not well understood. Fujiyoshi et al. [14] demonstrated antigen uptake into the lymphoepithelium after its incubation with horseradish peroxidase. They described the lymphoepithelium as part of a functional unit that includes the secondary follicle with the mantle zone facing the epithelium. It is not known if the formation of the secondary follicle can program differentiation of the overlying epithelium into highly specialized cells with ICAM-1 expression or if ICAM-1 is a result of an immediate up-regulation due to specific antigen exposure. Further studies are needed to determine if the adenoid epithelial cells can respond in an immediate fashion to antigen/allergen exposure by ICAM-1 up-regulation.

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References