Cytokine-Mediated Changes in PMN Adherence to Canine Tracheal Epithelial Cells*

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Airway inflammation is often accompanied by increased numbers of polymorphonuclear leukocytes (PMN) in

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airway secretions. These PMNs may contribute to airway epithelial cell injury in long-term and acute inflammatory airway disease. PMN toxicity to cells is dependent on close adherence of PMNs to the target cells.1 In endothelial cells, inflammatory cytokines, IL-1β and TNF-α, upregulate the specific PMN receptors to the endothelial cell surface.2,3 We hypothesized that cytokines released during acute and chronic inflammation would alter PMN adherence to the apical surface of airway epithelium.

**Materials and Methods**

Canine tracheal epithelial cells and human umbilical vein endothelial cells (HUVECs) were harvested, plated at a density of 3 x 10⁵ cells/filter, and grown to confluency on nitrocellulose filters that were precoated with human placental collagen. Cells were cultured in airway epithelial media containing Ham’s F-12, Dulbecco’s modified eagle media, and 5% fetal calf serum (FCS). All canine tracheal epithelial cells and HUVECs were primary cultures.

Whole blood was obtained from healthy human or canine donors. Neutrophils were isolated using dextran sedimentation and discontinuous gradients per Haslett et al. During the canine PMN isolation procedure, RBC lysis was performed using NH₄Cl 0.155 M buffered with NaEDTA and KHCO₃. The PMNs were counted, and the amount needed was labeled with ³⁵Cr.

The IL-1β, TNF-α, and INF-γ were diluted as recommended by their manufacturers. Cell monolayers were washed. New media containing the appropriate cytokine with bovine serum albumin (BSA) were placed on each monolayer and incubated at 37°C for the specified incubation time. Monolayers were washed again. The PMNs in FCS-free media were placed on each monolayer and incubated at 37°C for 30 min. Excess PMNs that did not adhere were washed from the monolayer by immersion. The PMNs adherent to the monolayer were lysed with NH₄OH and counted.

$$\% \text{ PMN adherence} = \frac{\text{cpm lyase}}{\text{cpm added}} \times 100$$

A leukocyte adhesion inhibitor assay was performed to look for a secreted epithelial cell product that caused decreased PMN adherence to cytokine-stimulated endothelial cells as previously described by Wheeler et al. Canine tracheal epithelial cells were grown in two plastic culture bottles. One was treated with IL-1β and both were washed with fresh FCS-free media. Fresh FCS-free media was left on the cells in each culture bottle for 16 h. The supernatants were collected. The PMNs were suspended in fresh or conditioned media from unstimulated or stimulated canine tracheal epithelial cells and placed on unstimulated or IL-1β-stimulated HUVECs for 30 min. The remainder of the experiment was performed as above.

**Results**

The optimal ratio of PMNs to epithelial cells was determined. Percent adherence remained constant for <1 to 2 PMNs per epithelial cell, but began to increase at 5 PMNs per epithelial cell. Scanning EM confirmed that at PMN-to-epithelial cell ratios of ≥5, adherence was no longer restricted to PMN-to-epithelial interactions, but involved clumps of PMNs. Therefore, all subsequent studies were conducted with PMN-to-epithelial cell ratios of 2:1.

We observed that adherence to the apical surface of untreated epithelia was consistently <10%. In contrast, PMN adherence to untreated endothelia was 30%.

As a positive control, HUVECs were treated with IL-1β at varying concentrations for 4 h with a resulting increase in adherence from 33.7 ± 2.6 to 55 ± 2.5%.

Primary canine tracheal epithelial cell monolayers were treated with IL-1β (5 U/ml), TNF-α (200 U/ml) or IFN-γ (100 U/ml) for 1 to 16 h. Incubation of canine tracheal epithelium with 5 U/ml IL-1β caused a time-dependent decrease in PMN adherence from 7.1 ± 1.8% with no treatment to 5 ± 0.4% after 16 h. Similarly, incubation of tracheal epithelium with 200 U/ml TNF-α decreased PMN adherence from 7.9 ± 0.8% with no treatment to 4.6 ± 0.3% after 16 h. However, incubation of tracheal epithelium with 100 U/ml IFN-γ did not change PMN adherence significantly (6.3 ± 0.3% with no treatment to 5.4 ± 0.4% after 16 h). Supernatants from IL-1β-treated epithelial cells did not inhibit the augmented PMN adherence to IL-1β-treated HUVECs, demonstrating that the decrease in adherence to treated epithelium was not mediated by a soluble factor secreted from the epithelial cells.

**Conclusions**

The baseline adherence of PMNs to the apical surface of epithelial cells is low. Exposure to IL-1β or TNF-α decreases PMN adherence to primary canine tracheal epithelial cells further. The PMN adherence is not affected by treatment with IFN-γ. This information suggests a modification of the PMN-to-epithelial cell receptor. Possibilities include increased production of an interfering substrate or downregulation of the epithelial cell receptors. This phenomenon may prove to be a protective response.

**References**


**Neutrophil Adhesion to Parainfluenza Virus-Infected Human Airway Epithelial Cells**

**Possible Contributions of ICAM-1-Dependent and ICAM-1-Independent Mechanisms**

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