

# Early Biochemical Markers of Inflammation in a Swine Model of Endotracheal Intubation

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**Background:** A common complaint after endotracheal tube use is sore throat, which may be due to abrasion, ischemia-reperfusion injury, or an inflammatory reaction. Few studies have evaluated localized tracheal inflammation as part of the response to intubation.

**Methods:** Inflammation of the trachea due to intubation was assessed in a swine model by following indicators of inflammation over time in the tracheal lumen. Repeated tracheal lavages proximal to the endotracheal tube cuff were performed, and recovered lavage was analyzed for cells, protein, lactate dehydrogenase, and cytokines.

**Results:** The baseline tracheal lavage samples contained 18% polymorphonuclear cells. These cells increased rapidly to 43% by 1 h. The polymorphonuclear cell increase from baseline was significant at 1, 2, and 4 h ( $P < 0.01$ ) after intubation. Tumor necrosis factor  $\alpha$ , interleukin  $1\beta$ , interleukin 6, and interleukin 8 increased over time, but only interleukin 6 increased significantly ( $P < 0.01$ ). Interleukin 6 was not detected at baseline or 1 h, but was detected at 2 h and increased significantly by 4 h. Neither lavage protein concentration nor lactate dehydrogenase activity increased over time.

**Conclusions:** These results demonstrate that inflammation does occur during tracheal intubation, even when markers suggest minimal tracheal damage. The dramatic elevation in polymorphonuclear cells, along with the increase in interleukin 6, suggests an inflammatory response to the endotracheal tube itself or to some aspect of the intubation process. A more complete understanding of the response of the tracheal tissues is important in improving the treatment of intubated patients.

TRACHEAL intubation is a necessary step in providing airway control during a variety of medical conditions. One of the most common complaints after surgery requiring tracheal intubation is sore throat,<sup>1–6</sup> and it has been reported to have an incidence between 6% and 90% even under optimal intubating conditions.<sup>7</sup> The roles in airway injury of all the components of tracheal intubation, including the response to the presence of the endotracheal tube (ETT), have not been elucidated. However, several factors have been postulated as being responsible for the airway injury, including mechanical trauma,<sup>8</sup> allergic reactions to the tube,<sup>9</sup> chemical injury,<sup>10,11</sup> and infection.<sup>12</sup>

There are many reports in the literature of postintubation tracheal injury, including inflammation, due to high ETT cuff pressure (ischemia-reperfusion injury).<sup>13,14</sup> In contrast, this study was designed to follow the inflammatory response of the trachea in the presence of the ETT with sustained cuff pressure, *i.e.*, no ischemia-reperfusion. The ETT even above the balloon is in direct contact with the tracheal mucosa and has been shown to result in changes in the trachea that suggest that inflammation may be involved in postintubation symptoms. The changes that have been observed are increases in blood flow (erythema),<sup>15–17</sup> elevation in laryngeal resistance (swelling),<sup>18,19</sup> and tracheal denudation,<sup>20</sup> all signs of localized tracheal injury. It is known that the airway epithelium is a natural barrier to a variety of pathogens (allergens, pollutants, bacteria). Airway epithelium is in direct contact with the ETT and probably plays a key role in any response to a potential stimulus. In this study, we sought to determine whether an inflammatory response of the tracheal tissue occurred in response to intubation by using tracheal lavage. Acute inflammation was documented by the influx of polymorphonuclear cells (PMNs) and the release of inflammatory cytokines tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin  $1\beta$  (IL- $1\beta$ ), interleukin 6 (IL-6), and interleukin 8 (IL-8) in swine.

## Materials and Methods

### Animal Preparation

We studied female domestic pigs (derived from White Yorkshire and Landrace strains) obtained from Oak Hill Genetics (Ewing, IL) using protocols approved by the Saint Louis University Animal Care Committee (St. Louis, MO). The animals were acclimated to their individual quarters (approved by the Association for Assessment and Accreditation of Laboratory Animal Care International) for 72 h after transportation. All pigs were judged to be clinically healthy by the staff veterinarian. Of the 26 pigs studied (21–45 kg), 2 were excluded because of difficulty of intubation (resulting in apparent tracheal hemorrhage).

The swine were sedated with an intramuscular injection of a mixture of ketamine (50 mg/ml), xylazine (50 mg/ml), tiletamine (50 mg/ml), and zolazepam (50 mg/ml) at a dose of 1 mg/kg body weight. The preoxygenated swine were anesthetized by inhaling isoflurane in oxygen (Forane®; Baxter Healthcare Corporation, Deerfield, IL) using a vaporizer specific for isoflurane (Ohmeda-Isotec 4; Ohmeda, Austell, GA), *via* a cone placed over the nose and mouth until corneal reflexes were

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undetectable and the measured exhaled isoflurane was at least 1.2% according to the respiratory gas monitoring device (Ohmeda 5250-RGM; Louisville, CO). The animals were placed in a supine position, the airway was visualized using a laryngoscope, 1 ml lidocaine was introduced into the trachea around the glottis, a tube guide was placed in the airway, and the ETT was inserted into the airway. The position of the ETT in the upper airway was fixed by securing the upper portion of the tube with the use of umbilical tape tied to the lower jaw (distal end being 26–28 cm from the angle of the mouth). ETT cuff pressures were set, monitored, and maintained using a calibrated pressure gauge (Posey Cufflator No. 8199; Posey Company, Arcadia, CA).

The following ventilator settings were used: tidal volume 10–12 ml/kg, respiratory rate 10–20 breaths/min, and positive end-expiratory pressure 5 to keep end-tidal carbon dioxide between 30 and 35 mmHg. An adult breathing circuit (Vital Signs, Totowa, NJ) was used to carry oxygen and inhalation anesthetic to the swine. After intubation, oxygen delivery was set at 1–2 l/min in combination with air to deliver 50–60% oxygen. Oxyhemoglobin saturation was maintained at 95% O<sub>2</sub> or higher as measured by pulse oximetry. Under aseptic conditions, an invasive arterial blood pressure monitoring catheter was placed either in the right femoral artery or in the auricular artery using percutaneous placement with a 22- to 24-gauge Angiocath catheter (Becton Dickinson, Sandy, UT) secured using silk 2-0 or clear tape. A peripheral intravenous line was placed on the ear lobe vein under aseptic conditions and secured with tape. Body temperature was monitored *via* rectal probe (Cole Parmer thermometer; Chicago, IL), and temperature was maintained at 36°–39°C using a warming blanket (Norm-temp®; Cincinnati Sub-Zero, Cincinnati, OH).

#### Tracheal Lavage

A standard polyvinyl chloride ETT (6.5 French; Mallinckrodt, Hazelwood, MO) was modified using a silicone, 14-French, dual-port Foley catheter attached to the exterior of the ETT, with the distal end of the Foley removed to expose the dual lumen 5 mm above the cuff. One of these dual-lumen tubes served as the lavage sampling port, and the other served as the injection port. The lavage of the trachea proximal to the upper portion of the inflated cuff was performed using 5 ml sterile normal saline with a push-pull technique that resulted in a return of 60–90% of the administered solution. We designed this tube to obtain a targeted tracheal sample in the experimental and control groups. In addition, four pigs were also lavaged under the same protocol using a regular Mallinckrodt Hi-Lo Evac® tube with sampling through the single port above the cuff to assess whether the attachment of the Foley might have affected our results. These same four pigs were maintained with 0 cuff pressure except for sampling, when the cuff was

inflated to 25 cm H<sub>2</sub>O for less than 40 s at a time. After the last tracheal lavage, the animals were killed by an intravenous injection of 75 mg/kg pentobarbital sodium (Sleep-away®, 260 mg/ml; Fort Dodge Laboratories, Inc., Fort Dodge, IA) in accordance with the American Veterinary Medical Association 2007 Guidelines on Euthanasia.

The recovered volume was measured and the cells were resuspended before an aliquot was removed for cell counts (hemacytometer) and for differential cell counting. The differential slides were made using cytocentrifugation of 100 µl whole lavage fluid in 100 µl Hanks Balanced Salt Solution with 1% bovine serum albumin followed by Diff Quick stain (IMEB, Inc., San Marcos, CA). Two hundred cells per cytocentrifuged sample were counted for each differential. The remaining lavage fluid was centrifuged at 1,500 rpm for 15 min at 4°C. The supernatant was divided into aliquots and frozen, and the cell pellet was resuspended in 400 µl lysis buffer (20 mM Tris pH 7.5/150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM-Na<sub>3</sub>VO<sub>4</sub>, 1 µg/ml leupeptin, 1 mM-PMSF) and frozen in aliquots.

#### Histology

At sacrifice after intubation, animal tracheas were filled with neutral buffered formalin for 10–15 min *in situ*. The tissues were then removed, and sections were immersed in neutral buffered formalin for 24 h before processing.

#### Assays

Bicinchoninic acid reagents (Pierce, Rockford, IL) were used for protein assay in triplicate on tracheal lavage supernatants and cell pellets based on an albumin standard curve. CytoTox 96 (Promega, Madison, WI) was used for the lactate dehydrogenase (LDH) assay on supernatants in duplicate using 50 µl undiluted cell-free supernatant. Cytokine levels in lavage cells and supernatants were determined in duplicate by enzyme-linked immunosorbent assay for porcine cytokines (R&D Systems, Minneapolis, MN) following the manufacturer's instructions without modification. The minimum detectable dose for all cytokines was 1–10 pg/ml. The average coefficient of variation of all assays was less than 5%.

#### Protocols

The tracheas of all 13 experimental animals were lavaged at 0, 1, 2, and 4 h. To determine the potential effects of repeated tracheal lavage, 3 pigs were intubated but were only lavaged once at 4 h. To determine the influence of the ETT *versus* the anesthetic agent on the inflammatory response, 3 pigs were ventilated with mask only for 4 h and then intubated to immediately obtain one lavage sample. To rule out effects of the

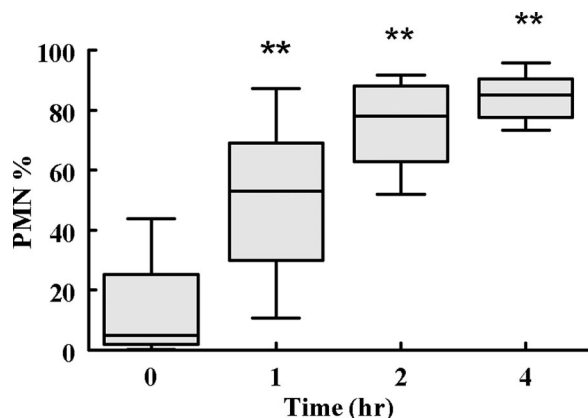


Fig. 1. Polymorphonuclear cells (PMNs) were measured over 4 h as a percent of total cells in tracheal lavage fluid at multiple time points. Data are presented as median with 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). \*\*  $P < 0.01$  compared with time 0 by Friedman and Wilcoxon test.

attached Foley, 4 pigs were intubated with a standard ETT and lavaged at all time points.

Because previous studies have shown a correlation of elevated cuff pressure with injury<sup>21</sup> and sore throat<sup>22</sup> in short-term tracheal intubation, we included both normal and high cuff pressures. The cuff pressure was set at insertion of the ETT and was maintained at that level for the duration of the experiment. In the experimental group, seven animals were studied with normal cuff pressures (25 cm H<sub>2</sub>O) and six animals were studied with high cuff pressures (2 at 50 cm H<sub>2</sub>O and 4 at 120 cm H<sub>2</sub>O). All control animals that had ETT were maintained at a cuff pressure of 25 cm H<sub>2</sub>O, except for the pigs without modified ETT, which had 0 cuff pressure.

#### Statistical Analysis

All data analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL). Data from multiple time points were analyzed by Friedman nonparametric two-way analysis of variance for repeated measures. Because doing these analyses with a split plot by cuff pressure revealed no difference between high- and low-pressure groups, the data for all pressures were pooled. Differences of each time point from time 0 were measured by Wilcoxon signed ranks test, followed by a Bonferroni correction for multiple comparisons, which placed the two-tailed  $P$  value for significance at 0.017. Differences between 0 and 4 h cell pellet data were assessed by Wilcoxon matched-pairs test, with a  $P$  value less than

0.05 considered significant. A Kruskal-Wallis test followed by a Mann-Whitney U posttest was used to determine significance in comparisons between multiple groups at a single time point. With a Bonferroni correction for multiple comparisons (each group *vs.* experimental group), a  $P$  value less than 0.017 was considered significant. Results in the text are expressed as mean  $\pm$  SEM, whereas all graphs show median and range data.

## Results

### Neutrophils in Tracheal Lumen

The influx of PMNs and plasma into tissue is the initial indicator of acute inflammation. Tracheal PMNs recovered in the lavage fluid of the experimental group after intubation demonstrated that PMNs were clearly recruited into the tracheal lumen in increasing numbers over time. The percentage of PMNs in the recovered lavage increased significantly at 1 h after intubation: The PMNs increased from 16% to 49.5% of all cells, and the total PMNs increased 221% (fig. 1) The PMNs increased in the tracheal lumen in a hyperbolic manner, with increases at all time points being significant relative to baseline. At 4 h, the PMNs were 85% of all lavage cells, with the absolute numbers of PMNs increasing 17-fold to  $1.02 \times 10^7$  per lavage (table 1).

### Lavage Composition

Cell differentials showed a predominance of macrophages and ciliated columnar epithelial cells at baseline ( $28 \pm 6\%$  and  $54 \pm 8\%$ , respectively). PMNs at baseline were  $16 \pm 4\%$  of cells, with the total number of recovered PMNs averaging  $6.15 \times 10^5$  in the lavage immediately after intubation. The remaining 2% of baseline cells were oral squamous cells.

The protein detected in tracheal lavage did not change significantly over time (data not shown), suggesting that the cytokines in the tracheal lavage probably were not caused by a progressive leakage from the plasma. The influence of the ETT on the epithelial cells in the tracheal wall was monitored by following LDH in the tracheal lavage. Nine of the 13 experimental pigs had their highest LDH at baseline, declining by 1 h and remaining below baseline value throughout the experiment (data not shown). These data support the absence of sustained tracheal cell death in these animals caused by the ETT.

Table 1. Total Polymorphonuclear Cell Number in Tracheal Lavage (Millions)

	Time 0	1 Hour	2 Hours	4 Hours
Mean $\pm$ SEM	0.65 $\pm$ 0.25	1.35 $\pm$ 0.55	1.88 $\pm$ 0.84	10.21 $\pm$ 4.87
Median (range)	0.02 (0–3.06)	0.49 (0.006–6.63)	0.84 (0.15–11.26)	1.33* (0.28–50.67)

n = 13 experimental pigs.

\*  $P < 0.001$  vs. time 0 by Friedman and Wilcoxon (each time compared with time 0).

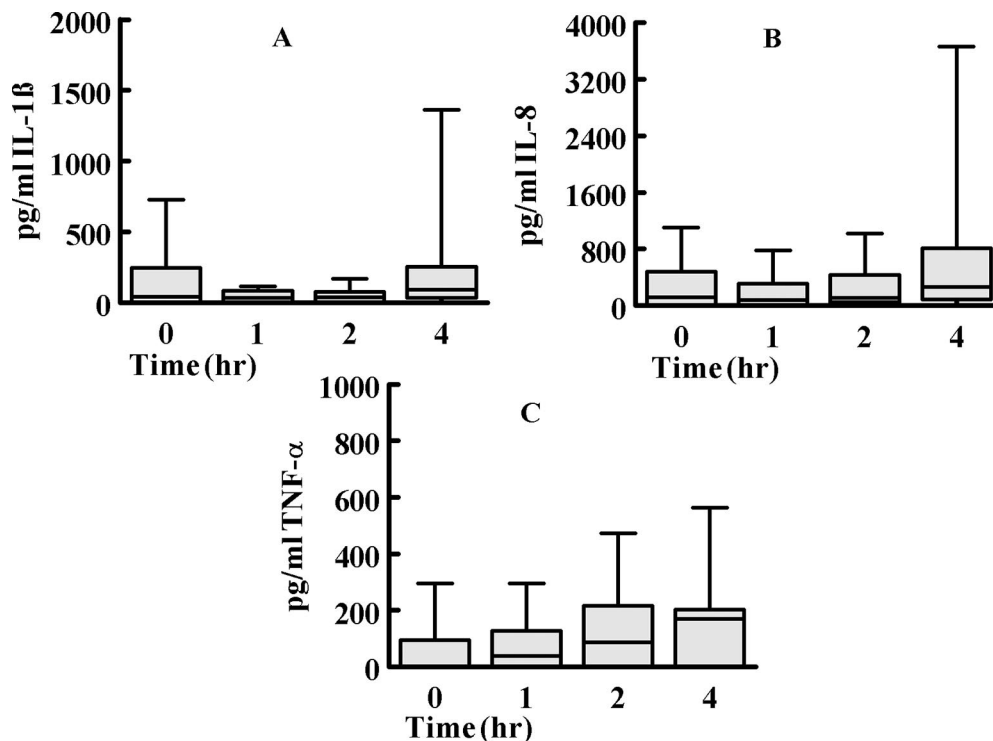


Fig. 2. Levels of interleukin 1β (IL-1β; A), interleukin 8 (IL-8; B), and tumor necrosis factor α (TNF-α; C) were measured by enzyme-linked immunosorbent assay in tracheal lavage fluid. Data are presented as median (pg/ml) with 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). Differences not significant by Friedman and Wilcoxon test.

Lavage Cytokines

Cytokines were assayed to determine their role in mediating the inflammatory response and the chemoattraction of the PMNs into the tracheal lumen. Cytokines were present in the tracheal lavages. IL-1β (141 pg/ml), IL-8 (304 pg/ml), and TNF-α (60 pg/ml) were measured at time 0 and tended to increase by 4 h after intubation, but none of these cytokines increased significantly from baseline (fig. 2). However, IL-6 was not detectable in any pig tracheal lavage until 2 h after intubation: The increase in lavage IL-6 (to 142 ± 48 pg/ml) was significant at 4 h (fig. 3).

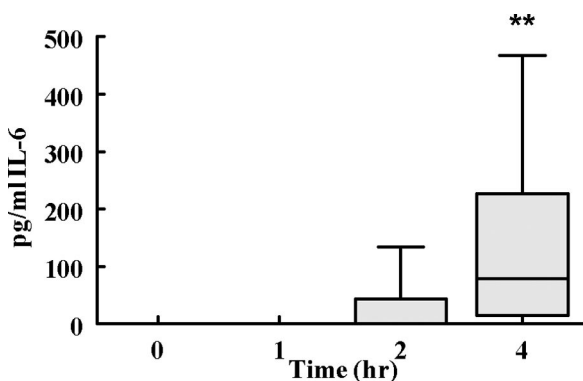


Fig. 3. Levels of interleukin 6 (IL-6) were measured by enzyme-linked immunosorbent assay in tracheal lavage fluid. Data are presented as median (pg/ml) with 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). \*\*  $P < 0.01$  compared with time 0 by Friedman and Wilcoxon test.

Effect of Cuff Pressure

In addition to finding the expected inflammation at elevated cuff pressures, we found that normal cuff pressures and even intubation and maintenance of an ETT with no cuff pressure also resulted in inflammatory changes. Influx of PMNs and appearance of IL-6 in the tracheal lavage fluid occurred in each intubated group regardless of pressure (table 2).

Table 2. Polymorphonuclear Cell Percent and Interleukin-6 Level by Cuff Pressure

Cuff Pressure	Time 0	1 Hour	2 Hours	4 Hours
PMNs, %				
0	11 (7–18)	30 (19–50)	58 (43–88)	80 (47–88)
25	5 (1–45)	58 (9–90)	65 (51–90)	88 (78–96)
50	11 (2–20)	53 (51–55)	72 (57–87)	81 (73–88)
120	21 (0–26)	42 (30–68)	66 (5–98)	81 (75–94)
IL-6, pg/ml				
0	0	0	0	14 (0–88)
25	0	0	0 (0–143)	47 (0–470)
50	0	0	17 (0–34)	224 (79–369)
120	0	0	0 (0–71)	56 (0–114)

Control pigs (n = 4) were maintained at a cuff pressure of 0 cm H<sub>2</sub>O, and experimental pigs were maintained at cuff pressures of 25 cm H<sub>2</sub>O (n = 7), 50 cm H<sub>2</sub>O (n = 2), or 120 cm H<sub>2</sub>O (n = 4) for 4 h of intubation with a modified endotracheal tube. Polymorphonuclear cells (PMNs) are expressed as a percent of total cells, and interleukin 6 (IL-6) is expressed in pg/ml by enzyme-linked immunosorbent assay in tracheal lavages at four time points. Values are median ± range.

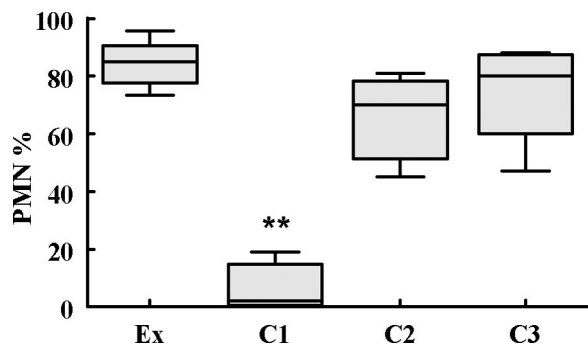


Fig. 4. Polymorphonuclear cells (PMNs) were measured as a percent of total cells at 4 h in tracheal lavage fluid of experimental pigs that were intubated for 4 h with lavage samples at time 0, 1, 2, and 4 h (Ex,  $n = 13$ ), pigs that had mask-only ventilation for 4 h before intubation and lavage (C1,  $n = 3$ ), pigs that had 4 h of intubation but no lavage until 4 h (C2,  $n = 3$ ), and pigs that were treated exactly the same as experimental pigs but had an endotracheal tube with no attached Foley and had 0 cuff pressure (C3,  $n = 4$ ). Data are presented as median (percent PMNs) with 25th–75th percentiles (boxes) and 5th–95th percentiles (whiskers). \*\*  $P < 0.01$  versus Ex by Kruskal–Wallis and Mann–Whitney U test.

#### Cell Pellets

To determine possible sources of the cytokines in the lavage fluid, measurements were made on cytokine levels in the cells recovered from the tracheal lavage. The lysed cell pellets from time 0 and 4 h lavages were assessed for cytokines IL-8, IL-6, and TNF- $\alpha$ . The total number of cells washed out at time 0 was  $2.52 \pm 0.67 \times 10^6$ , and at 4 h, the number was  $1.19 \pm 0.56 \times 10^7$ . All cell pellets had measurable IL-8 ( $1,444 \pm 494$  and  $6,662 \pm 2,576$  pg/ml at 0 and 4 h, respectively;  $P < 0.01$ ). IL-6 in cell pellets was  $47 \pm 27$  pg/ml at time 0 and  $96 \pm 37$  pg/ml at 4 h (not significant), and measurable TNF- $\alpha$  was found in only one cell pellet at time 0 and three cell pellets at 4 h.

#### Control Experiments

The results of the experimental group versus all control groups in percent PMNs in lavage at 4 h is shown in figure 4. Because we performed repeated lavages, which can be associated with increased PMN recruitment in the

alveolar space,<sup>23</sup> we compared PMN percent in lavage at 4 h between intubated pigs that had been repeatedly lavaged (experimental protocol) and pigs that had not been lavaged until 4 h after intubation. These groups did not differ significantly in PMN percent, suggesting that repeated lavage was not the causative factor in PMN recruitment. The effect of anesthetic and the presence of the ETT on the response were evaluated by providing the isoflurane to three pigs by mask for 4 h and then sampling immediately by intubation. The mask-only group at 4 h had PMN levels comparable to those of all pigs at baseline but significantly different from experimental pigs at 4 h, suggesting that the PMN influx was related to the ETT and not the anesthetic. The four pigs that had a standard ETT with no Foley but were otherwise treated as experimental pigs did not differ significantly from the experimental group in PMN percent at 4 h.

#### Histology

Histologic observation of randomly selected tracheas at the end of the experiment showed no gross signs of injury: The tracheal epithelium was intact and not detached from the basement membrane (fig. 5). In the experimental tracheas, subjectively there was a clear increase in PMNs present in the tracheal lumen and in the epithelium and tissue adjacent to the epithelial cells.

#### Discussion

We tested the hypothesis that inflammation is part of the local response to tracheal intubation (includes placement and response over time to a foreign object, the tube). We have demonstrated that intubation results in inflammation of the upper trachea and that it can be assessed by measuring inflammatory markers in the tracheal lumen by the use of lavage. The principal findings of our study were that after intubation, PMNs increased both in percentage and in absolute numbers, and the acute phase cytokine IL-6 increased in the lavage. The

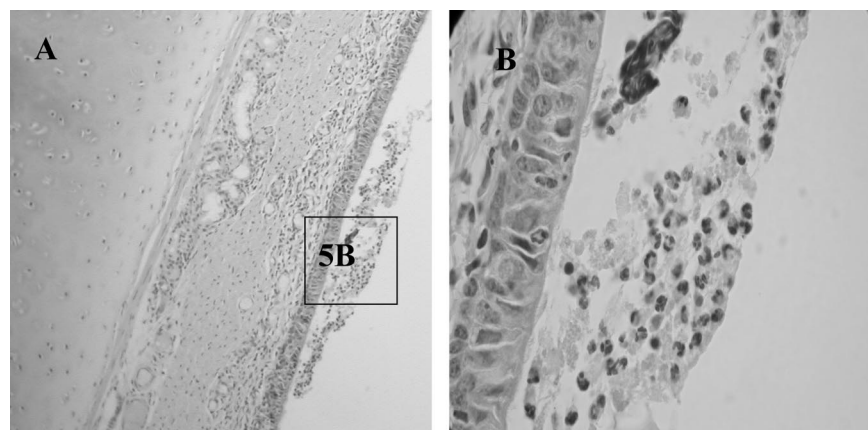


Fig. 5. Hematoxylin and eosin–stained histology of pig trachea after 4 h of intubation. (A) Image demonstrates intact epithelial layer at 2 cm distal to the larynx. Magnification  $\times 100$ . (B) Image of epithelium and lumen enlarged from area indicated in A. Demonstrates presence of polymorphonuclear cells in epithelium and in the mucous in tracheal lumen. Magnification  $\times 1,000$ .

previous reports of the presence of an ETT resulting in increased tracheal swelling<sup>18</sup> and a 30-fold increase in blood flow<sup>15-17</sup> also support the conclusion that intubation results in the classic symptoms of inflammation. Individually, the presence of PMNs, IL-6, swelling, and hyperemia would not support the hypothesis that inflammation is part of the response to tracheal intubation. However, when viewed collectively, they demonstrate that inflammation is part of the response.

The baseline levels of PMNs in the tracheal lavage (16%) were higher than has been reported in the distal airway/alveoli (0-3%).<sup>23</sup> This might be expected because the upper trachea is the last barrier in the host defense of the lung. The elevated PMNs may be due to the presence of IL-8 in the tracheal lumen, which could function as a sustained chemoattractant in the airway. Caution should be used in interpreting the lavage concentrations of IL-8 because the unknown volume of the compartment being lavaged makes the calculation of the effective tissue mediator/marker difficult.

The influx of PMNs into a tissue space is due to the release of chemotactic factors, usually by monocyte-derived cells. The trachea contains a wide variety of cell types that could have been stimulated to produce the chemotactic agents. IL-8 has been shown to be produced by tracheal epithelial cells,<sup>24,25</sup> airway smooth muscle cells,<sup>26</sup> and airway macrophages.<sup>27</sup> The proinflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-8 were observed in the tracheal lavages at all time points. The concentrations of these cytokines tended to increase gradually with the time of intubation; however, none of these cytokines was correlated with the PMN influx. The large increases in PMNs in the lavage occurred before the significant increase in IL-6. Therefore, the chemotactic factor has yet to be identified.

It is not clear which aspect of tracheal intubation results in this inflammation. Tracheal intubation can result in the introduction of oral organisms (minor local infection), abrasion of the tracheal mucosa (direct injury), a reaction to the tube material, and tracheal trauma/irritation caused by ETT cuff pressure.

The introduction of a limited number of oral epithelial cells and some oral flora into the trachea could have initiated the inflammation observed. We observed bacteria in the lavages in varying numbers throughout the experiment. It is unclear what numbers of bacteria would be necessary to induce inflammation due to infection from oral contamination. Therefore, we cannot rule out infection as being a stimulus for inflammation in these experiments. As for direct injury, the submucosal area of the trachea is highly vascular, and any disruption due to trauma to the tissue would probably result in increased protein in the lavage. In these experiments, we observed no significant change in the protein concentration in the lavage, suggesting limited trauma to the tracheal tissue. However, the large amount of LDH

present in the baseline lavage indicates that significant cellular disruption does occur with even routine intubation. LDH decreases after the initial sample but remains detectable, perhaps because of prolonged effects of the intubation process.

The tracheal response to a foreign object (in this case the ETT) is more difficult to assess because of the problems with identifying a trigger for the response. Di-(2-ethylhexyl)-phthalate, an intrinsic component of the ETT, or similar compounds may serve as potential trigger released by the ETT. There is evidence of human PMN activation after exposure to di-(2-ethylhexyl)-phthalate.<sup>28</sup> IL-6 has been reported to mediate the innate immune response under a variety of conditions.<sup>29</sup> We have observed that IL-6 is significantly increased with duration of exposure to the ETT, which may suggest that innate immunity is involved in this response. The other possibility is a synergistic activity of all these factors, resulting in an inflammatory response that is magnified over time. The exact components of the inflammatory cascade need to be elucidated.

To evaluate the contribution of the cuff pressure, we conducted this series of experiments using a range of cuff pressures from 25 to 100 cm H<sub>2</sub>O. Cuff pressures of 100 cm H<sub>2</sub>O have been shown to cause injury to the tracheal mucosa in contact with the cuff.<sup>15</sup> We found that cuff pressure did not significantly alter the response of any of the indicators of inflammation. The control group of pigs studied with 0 cuff pressure confirmed this observation. Because cuff pressure had no apparent effect on these indicators of inflammation, it is not likely that the inflammation observed during these relatively short-term intubations was due to the cuff.

The use of repeated lavages was demonstrated to have an insignificant influence on the results of this study (fig. 4). Previous studies of the proinflammatory effects of a single bronchoalveolar lavage have not been conducted in the presence of another inflammatory stimulus.<sup>23</sup> The use of repeated lavages to follow the time course of the response requires careful analysis. Not only are the observations obtained with the second lavage influenced by the previous lavage, but the potential inflammatory stimuli may also be diminished by the initial lavage. The lavage data at any time point are influenced by the previous lavages: If the tracheal tissues were quiescent, the component found in the lavage would decrease exponentially. This is because the amount of the lavage recovered averaged 80%, leaving 20% of all indicators in the trachea. In some cases, the lavage concentrations did decrease predictably with subsequent lavages (LDH). However, most indicators remained constant or increased (all measured cytokines). The non-decreasing pattern in the lavage indicates an ongoing rate of production.

The differential analysis of the lavage cells indicates that at 4 h, the total number of cells in the pellet increased 5-fold from baseline. Also, the composition of

the lavage cell pellets was altered in that PMNs increased from 16% to 85%. The significant increase in IL-8 in the pellets was not accompanied by a significant increase in IL-8 in the lavage. Quantitative analysis of the IL-8 concentrations in the cells and the lavage indicate that there is a gradient of approximately 100-fold between the intracellular concentrations and the concentrations expected in the tracheal fluid. Therefore, we assume that the cells present in the tracheal lumen as recovered in the lavage could be one of the sources of IL-8 observed in the lavage. This role of the PMN is supported by the findings of Cassatella *et al.*,<sup>30</sup> who reported that PMNs have been shown to produce IL-8. The recovered cells were probably not a source for the TNF- $\alpha$  observed in the lavage because TNF- $\alpha$  was seldom measured in a cell pellet.

Interleukin 6 was present in three of the cell pellets at baseline, but no IL-6 was detected in any lavage fluid at that time point. Analysis of the concentration gradients between the lavage and the cell pellet at 4 h indicates a similar concentration gradient from cells to lavage for IL-6 as that observed for IL-8. It is not clear what cell types were responsible for the IL-6 in the lavage. However, because the PMN number increased 25-fold and the IL-6 increased only 2-fold, it is unlikely that PMNs were the source of IL-6. The data indicate that the most likely cellular source for IL-6 in the lavage was the tracheal epithelium. Our data indirectly support the findings of Schroder *et al.*,<sup>31</sup> who reported that PMNs do not produce IL-6.

Our study was conducted in swine, which provided us with the ability to reproduce the general conditions seen in human subjects during tracheal intubation. However, the possibility of species variation could account for some differences when the same experiments are conducted in humans. The inability to assess subjective discomfort (dysphonia, sore throat) in these animals represents a limitation of our study. We conducted our experiments during a preset amount of time, and it may be necessary to extend the time of exposure to the ETT. Finally, the possibility that inflammation is not only a local reaction to a foreign object but also a very early reaction secondary to seeding of the trachea by bacteria, trauma, or innate immune response is an area that requires more investigation.

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