Stress Hormone Epinephrine Increases IgA Transport across Respiratory Epithelial Surfaces

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BACKGROUND: Secretory immunoglobulin A (sIgA) is the principle antibody produced at the respiratory surface. Respiratory sIgA levels are increased early after injury in both human and laboratory animals; the mechanisms are uncertain. Stress hormones, including epinephrine (Epi) and norepinephrine (NE), increase early after injury. In addition, respiratory epithelial cells are known to be responsive to β_2-agonists. We therefore studied the effect of Epi, NE, and albuterol on IgA transport in vitro.

STUDY DESIGN: Calu-3 respiratory epithelial cell monolayers grown in a 2-chamber cell culture system were treated for 24 hours with Epi, NE, or albuterol (10^{-6}M). Dimeric IgA was added to the basal chamber of Calu-3 cells and IgA transcellular transport was indexed by recovery of SIgA in the apical chamber by enzyme-linked immunosorbent assay. In separate experiments, *Klebsiella pneumoniae* (10^5 colony-forming units/mL) was added to the apical chamber of treated Calu-3 cell monolayers and bacterial passage across Calu-3 cells was determined by bacterial recovery from basal chamber media. Calu-3 cells not treated with Epi, NE, or albuterol served as control. Cell monolayer integrity was confirmed by transepithelial electrical resistance.

RESULTS: Calu-3 cells treated with Epi led to a significant increase in sIgA transport, this was associated with an increase in polyimmunoglobulin receptor expression. Calu-3 cells treated with NE or albuterol showed no statistical difference compared with control. Only cells treated with Epi led to a significant increase in pro-inflammatory cytokine expression and decrease in bacterial passage.

CONCLUSIONS: Epinephrine is likely an early upstream signal in the enhanced IgA response at respiratory surfaces after injury. (J Am Coll Surg 2014;218:450–458. © 2014 by the American College of Surgeons)

Pneumonia is a common cause of morbidity and mortality after trauma and hemorrhagic shock. The CDC defines ventilator-associated pneumonia as pneumonia in patients who had a device to assist or control respiration continuously through a tracheostomy or by endotracheal intubation within the 48-hour period before the onset of infection, inclusive of the weaning period. Ventilator-associated pneumonia is associated with a 25% to 50% mortality rate.[1,2] The economic impact of ventilator-associated pneumonia is substantial, with costs ranging from $19,633 to $28,538.00 per patient with ventilator-associated pneumonia.[3] Therefore, a lot of effort has been made to reduce the incidence of pneumonia by applying standards of care in critically ill patients who are intubated and who are represented by the ventilator care bundles.[4,5] Despite all of these efforts, ventilator-associated pneumonia after trauma remains a challenge, so investigators are now aiming toward prevention, and to do that it is key to understand the pathophysiology behind lung protective mechanisms.

The lungs are protected by a mucosal barrier usually present in the upper airway, and are also protected by a more complex immune system represented by the innate and adaptive immune system. Immunoglobulin (Ig) A is the major immunoglobulin at mucosal surfaces, including the respiratory airway. Immunoglobulin A is mainly concentrated in the upper airway, and IgG is found mainly in the lower airway, however, the latter can cross to the luminal surface by means of diffusion. Previous clinical investigators have studied the relationship between IgA and pneumonia, correlating reduced IgA with higher incidence of pneumonia.[6]

Immunoglobulin A is produced at the basolateral surface of the respiratory epithelial cells by plasma cells and is then transported to the epithelial cells by means of a special receptor expressed in the basolateral surface, known as polyimmunoglobulin receptor (pIgR). Once

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Abbreviations and Acronyms

dIgA = dimeric immunoglobulin A
DMEM = Dulbecco's Modified Eagle medium
ELISA = enzyme-linked immunosorbent assay
Epi = epinephrine
Ig = immunoglobulin
IL = interleukin
NE = norepinephrine
pIgR = polyimmunoglobulin receptor
sIgA = secretory immunoglobulin A
TEER = transepithelial electrical resistance
TNF = tumor necrosis factor

the complex is internalized and reaches the luminal surface, pIgR becomes unstable and is cleaved, releasing a small protein known as the secretory component. Dimeric IgA along with secretory component forms secretory IgA (sIgA), which protects the airway through various mechanisms, including bacterial exclusion, which prevents bacterial attachment to the mucosal surface. As a result, bacteria cannot attach to the respiratory surface and will not cause infection.[7,8]

Several known factors affect the concentration of IgA at the mucosal surface. We were able to demonstrate in our laboratory, using an in vitro model, that IgA transport was significantly increased by stimulation of toll like receptor−4 or estradiol.[9,10] Recent clinical and laboratory studies demonstrated that early in trauma, there is an increase in IgA transport, which was accompanied by an increase in pro-inflammatory cytokine recovery from bronchoalveolar lavage samples after trauma. In a different series of experiments, the pro-inflammatory cytokine receptors were blocked, which led to abrogation of the airway IgA response to injury. They concluded that early after trauma, there is an increase in pro-inflammatory cytokines that leads to an increase in IgA transport.[11-13] On the other hand, studies also found that early after injury and hemorrhagic shock, there is an increase in stress hormones and neurotransmitters that can alter cytokine release in the early phase after trauma.[14]

We hypothesized that early after trauma, there is an increase in stress hormones that alter pro-inflammatory cytokine release, which eventually leads to an increase in IgA transport. In addition, respiratory epithelial cells are known to be responsive to β_2 agonists. We therefore conducted an in vitro study with the following objectives: first, we studied the effect of epinephrine (Epi), norepinephrine (NE), and albuterol on IgA transport across cultured human respiratory epithelial cells in vitro (Calu-3 cells); our second objective was to examine if Epi, NE, or albuterol had any effect on pro-inflammatory cytokines; and, lastly, we examined whether induced modulation of IgA transport effects bacterial passage across Calu-3 cells.

METHODS

Respiratory epithelial cells

Calu-3 cells were obtained from American Type Culture Collection and routinely cultured with Dulbecco's Modified Eagle medium (DMEM) containing 10% fetal bovine serum, 4.5 g/L glucose, and 1% antibiotic

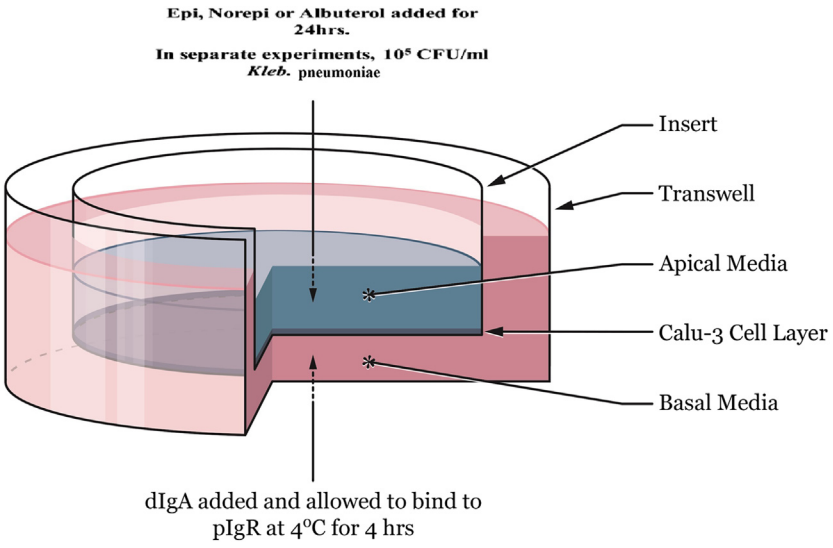

Figure 1. Diagram of experimental design. A schematic representation of the 2-chamber cell culture system used in the design of all experiments. CFU, colony-forming unit; dIgA, dimeric immunoglobulin A; Epi, epinephrine; pIgR, polyimmunoglobulin receptor; Norepi, norepinephrine.