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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.
Extracellular purines in vascular endothelial barrier preservation

Alexander D Verin, PhD
1. Clinical and physiological importance of lung vascular barrier

2. How to measure vascular permeability in vitro and in vivo

3. Mechanisms regulating endothelial permeability

4. Extracellular purines and endothelial barrier

5. Mechanisms of purine-induced EC barrier preservation
Lung Vascular Barrier

- Comprise of 3 major components: endothelium, basement membrane and epithelium

- Regulates exchange of solutes and fluid between blood vessels and alveoli

- Compromise of vascular barrier due to Inflammatory or toxic events results in increased permeability pulmonary edema (fluid accumulation) into the lung, which is a cardinal feature of acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS)

- ALI/ARDS leads to impaired gas exchange and may cause respiratory failure.

- There is no standard treatment for permeability pulmonary edema only ventilation strategies
The Normal Alveolus (Left-hand side) and the Injured Alveolus in the Acute Phase of ALI (Right-hand side)

- Cytokines
- Neutrophils
- Widened Interstitium (leakage of protein rich fluid)
- Gap Formation

Alveolar edema fluid flooding

Ware & Matthay, NEJM, 2000
Sepsis and pneumonia are the most common, causing about 60% of cases.
# ALI & ARDS - Incidence & Mortality in the US Alone

<table>
<thead>
<tr>
<th>Variable</th>
<th>Acute Lung Injury</th>
<th>ARDS</th>
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<tbody>
<tr>
<td>Cases — no.</td>
<td>1,113</td>
<td>828</td>
</tr>
<tr>
<td>Crude incidence — no. per 100,000 person-yr</td>
<td>78.9</td>
<td>58.7</td>
</tr>
<tr>
<td>Age-adjusted incidence — no. per 100,000 person-yr†</td>
<td>86.2</td>
<td>64.0</td>
</tr>
<tr>
<td>Mortality (95% CI) — %</td>
<td>38.5 (34.9–42.2)</td>
<td>41.1 (36.7–45.4)</td>
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<tr>
<td>Estimated annual cases — no.†</td>
<td>190,600</td>
<td>141,500</td>
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<tr>
<td>Estimated annual deaths — no.†</td>
<td>74,500</td>
<td>59,000</td>
</tr>
<tr>
<td>Estimated annual hospital days — no.†</td>
<td>3,622,000</td>
<td>2,746,000</td>
</tr>
<tr>
<td>Estimated annual days in ICU — no.†</td>
<td>2,154,000</td>
<td>1,642,000</td>
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* ARDS denotes acute respiratory distress syndrome, and CI confidence interval.
† U.S. estimates, age-adjusted to the 2000 Census, are shown.
Vascular permeability pathways

Permeability across endothelial and epithelial cell monolayers can involve transcellular, paracellular or both pathways. However, the majority of trafficking occurred through paracellular pathway.
Method for assaying endothelial barrier properties \textit{in vitro}

A. Electrical Cell-substrate Resistance Sensor System (ECIS)

- Endothelial Cells
- Culture Medium
- Large Gold Counter Electrode
- Gold Electrode
- Lock-in Amplifier
- Computer
- 1 M\(\Omega\)
- 1V AC Voltage

B. Resistance Tracing

- Vehicle
- Thrombin

Agonist-induced decrease in transendothelial electrical resistance (TER) reflects EC barrier compromise
Vascular leakage is primarily caused by an increase permeability of the endothelium

(Michel and Curry, 1999; Renkin, 1985).
The endothelial cell barrier is regulated by contractile and tethering mechanisms whose effects are critically dependent upon cytoskeletal components.
Bioactive agonists, growth factors, cytokines and mechanical forces (high shear stress or cyclic stretch), as well as activated leukocytes, serve to activate vascular endothelium. This produces cellular contraction, and increased passage of fluid and cells through intercellular spaces into the interstitial to initiate organ dysfunction.
Factors involved in maintaining endothelial integrity/restoration

These include low levels of shear stress, the negatively charged glycocalyx, and barrier protective molecules released by circulating platelets such as sphingosine 1-phosphate
Extracellular purines such as ATP, and its degradation product, adenosine, are important vascular mediators.

They are readily present in the surrounding EC micro-environment in vivo, and can be released into extracellular fluids under pro-inflammatory conditions from several cell sources including endothelium.

Recently the therapeutic potential of purinergic agonists in the treatment of cardiovascular and pulmonary diseases has been studied.

In the USA, adenosine is clinically used for tachycardia treatment.

Recent data implicate the involvement of extracellular purines in EC barrier enhancement/protection.
Extracellular purine-induced signaling in endothelium

**Blood**

- ATP → ADP → AMP → Adenosine

**Apical**

- Ectonucleotidases
- P2Y2
- P2Y13

**Basolateral**

- Ectonucleotidases
- A2A
- A2B

- ATP → ADP → AMP → Adenosine

**Endothelial cell**

**Basement membrane and alveolar epithelium**
Effect of purinergic stimulation on EC permeability

Dose-dependent effect of ATP on TER

Agonists of P2 receptors (50 μM each, 30 min) increase TER
Effect of ATP on cell-cell junctions

ATP Increases the Surface Area of the Cell-Cell Interface

Quantification of the surface area of the cell-cell interface. The percentage of total cell surface area occupied by VE-cadherin-labeled cell-cell junctions was calculated for 20 cells in each group (* p < 0.001 compared to control).
Adenosine enhances and restores EC barrier *in vitro*
Effect of adenosine post-treatment on vascular permeability and inflammation in murine model of LPS-induced ALI

Adenosine (i.v., 100 µM in blood, added 3 hr after LPS) significantly attenuates LPS (i.t., 0.9 mg/kg)-induced vascular leak and inflammation in mice.
Histological assessment of the effect of adenosine on LPS-induced lung inflammation and injury.

H&E staining (A) and lung injury score (B) demonstrate prominent lung inflammation in mice exposed to i.t. LPS compare to vehicle. Treatment with adenosine attenuates LPS-induced response.
Adenosine attenuates LPS-induced pro-inflammatory cytokine production in murine model of ALI.
Summary (1):

1. Extracellular purines, ATP and adenosine, enhances and restores endothelial barrier in vitro

2. Extracellular purines protect lung vascular barrier and reduce inflammation in murine model of LPS-induced lung injury
Mechanisms of purine-induced EC barrier preservation

Effect of purinergic receptors depletion on EC barrier enhancement induced by extracellular purines

P2Y1 is involved in EC barrier regulation

A2A, but not A2B receptor is involved in EC barrier regulation
Extracellular purines enhance endothelial barrier via G protein-coupled mechanism

Depletion of Gαq and i2 attenuates ATP-induced barrier enhancement (A, B), whereas depletion of Gαs but not Gαq or i2 involves in adenosine-induced effect (C). Collectively, these data demonstrate that ATP and adenosine activate distinct G-protein-mediated pathways.
ATP –induced EC barrier enhancement involved PKA activation

A. ATP increases PKA activity.
B. Inhibition of PKA attenuates EC ATP-induced EC barrier enhancement
C. ATP does not increases cAMP in EC. In contrast, adenosine agonist, NECA significantly increases cAMP suggesting distinct signaling involved in ATP and adenosine-induced PKA activation.
Role of MLC phosphorylation in the regulation of EC barrier

Actomyosin Contraction

Myosin Light Chain (MLC) phosphorylation is crucial for the regulation of EC barrier function. MLC phosphorylation by Myosin Light Chain Kinase (MLCK) facilitates cellular contraction and stress fiber formation, leading to barrier dysfunction. Myosin-associated phosphatase (MLCP) by dephosphorylating MLC may be involved in EC barrier preservation.
Effect of MLCP depletion on adenosine-induced EC barrier enhancement

A, B. Depletion of catalytic MLCP subunit (CS1β), but not CS1α-control attenuates adenosine-induced EC barrier enhancement. Depletion of MLCP regulatory subunit (MYPT 1) demonstrates the same effect (C)
Summary (2):

1. ATP and adenosine enhances EC barrier by activation of different signaling

2. Purine-induced EC barrier enhancement involves activation of protein kinase A and myosin phosphatase
CONCLUSION

- Increase of extracellular purines
- Receptors activation
- Trimeric G-proteins engagement
- Small G-proteins activation
- Activation of regulatory enzymes
- Changes in phosphorylation
- Subcellular localization of cytoskeletal targets
- Lung injury prevention

**Cytoskeletal Targets**

**EC barrier enhancement**
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