The Role Of The Epithelium In Sensitisation

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Outline of Talk

- The Human Airway
- The Bronchial Epithelial Barrier
- The Epithelial Barrier in Asthma
- Models of the Epithelial Barrier
Why Do We Need Epithelial Barriers?

• Multicellular organisms require tissue compartmentalization to support specialized functions.

• Cells that cover the external surface and line the internal compartments must form barriers to define boundaries and prevent unrestricted exchange of materials.

• The nature of the cells forming a particular barrier reflects the specialized function at that site.

• The functions of epithelia are related in some way to interaction between the internal and external environments of the body: eg. nutrition, gas exchange, excretion, or the intrusions and extrusions required for reproduction.
The Human Airway & Structure Of The Bronchial Wall

- Larynx
- Primary bronchi
- Secondary bronchi
- Tertiary bronchi
- Bronchioles
- Cardiac notch

Healthy airway
- Dendritic cell
- Macrophage
- Goblet cell
- Epithelial cells
- Bronchial epithelium
- Lamina densa
- Basement membrane
- Lamina reticularis
- Fibroblasts
- Smooth muscle cells
- Serous gland
- Mucous gland
- Cartilage/adventitia

Structure Of The Bronchioles and Alveoli
The Epithelial Barrier
The Bronchial Epithelial Barrier

2) Physical Barrier
- structurally and functionally polarized barrier maintains tissue integrity
- regulates transcellular and paracellular permeability (fence and gate tight junction)
- mucociliary escalator

3) Immunological Barrier
- immunoglobulins
- immune surveillance (PAMPs, DAMPs/alarmins)
- Interaction and recruitment of effector immune cells and APCs in the lamina propria

1) Chemical Barrier
- mucus
- anti-oxidants
- host defence peptides

Epithelium plays a key role in tissue homeostasis

(1) The Chemical Barrier

*detoxifies noxious substances and traps particulates*

**Anti-oxidants**
- including glutathione, ascorbic acid and anti-oxidant enzymes

**Mucus**
- Produced by goblet, serous and clara cells of the airways (>191 proteins)
- Complex mixture of >191 proteins but mainly composed of glycoproteins called Mucins (MUC5AC, MUC5B)
- Mucins are highly charged molecules which cross link to form a viscoelastic gel which traps particulates and microbes and prevents dehydration of epithelial surface

**Other** anti-microbial proteins including complement, immunoglobulins, surfactant proteins
(2) The Physical Barrier

A) Mucociliary Escalator
- Comprises mucus and cilia in pericilliary liquid (airway surface liquid)
- Mucus traps particles
- Beating of cilia removes particles from airways

B) Epithelial Junctions
- Structurally and functionally polarized barrier to maintain barrier integrity
- Regulates solute permeability (charge and size) and hydration of the airway surface liquid
- Fence (tight junctions) and gate (paracellular) functions
Epithelial Tight Junctions

- Tight junction comprised of proteins involved in apical-basolateral cell polarity, Signal transduction, Binding to cytoskeleton
- Semi-permeable diffusion barrier which restricts passive diffusion of molecules based on size and charge

Occludin
1st indentified and regulate TJ permeability to different sized particles (small hydrophilic proteins)

Claudins
Charged proteins which regulate TJ permeability to charged particles/ions (aqueous pores, ion selective transport Na, Cl)

Junctional adhesion molecules (JAM) function as adhesion proteins and regulate leukocyte transmigration
Dense network of immunological cells provide innate and adaptive barrier to foreign particles including bacteria, viruses, and allergens lie underneath basement membrane of epithelial barrier.

- Both Humoral Arm and Cellular Arm
- Humoral Arm includes Immunoglobulins (IgA), lysozymes, surfactant proteins, lectins and defensins
- Cellular Arm – macrophages, epithelial cells
- Non-specific i.e. innate
Evidence for epithelial cells in pivotal role in the induction of innate immunity

Express Pattern recognition receptors including Toll like receptors (TLRS) which when activated by PAMPs release an array of cytokines (TSLP) and chemokines

Express damage associated molecular patterns (DAMPs)

Release mediators in response to viruses, bacteria and allergens

Can activate underlying immune cells and recruit immune cells
**Dendritic cell**
- Next slide

**Mast cell**
- Epithelial cells express the pro-survival and chemotactic MC-specific mediator stem cell factor
- BEC and MC co-culture results in immature MC proliferation and suppression of mature MC activation
- MCs increased in epithelial layer in asthmatic subjects and have activated phenotype

**T cell**
- Increased in asthmatic airways
- Epithelial cell contribute to T cell survival and recruitment through release of TARC

**Eosinophils**
- Increased in asthmatic airways
- Epithelial cells release eotaxin, RANTES, increased eosinophil survival following co-culture
Epithelial Cell & Dendritic Cell Interactions

• DCs lie in close proximity to epithelial cells and sample Ag in luminal surface through expression of tight junction proteins

Effect of epithelium on DC precursors (monocytes)

• release MCP1 and MCP4 which attract DC precursors (Stellato et al 1997)
• induce monocyte differentiation into DCs which possess higher levels of CD80, CD40 and HLA-DR, increased Ag capture and processing and reduced capacity to induce T cell cytokine secretion via a type I IFN mechanism (Rate et al 2008)
• Inflammatory (IL-1β) activation of epithelial cells supports monocyte differentiation to DCs through release of IL-15 and generate DCs which induce T cell proliferation (Regamey et al 2007)

Effect of epithelium on immature DCs

• release CCL20 which attracts immature DCs via CCR6 (Pichavant et al 2005; Thorley et al 2005)
• Diesel Exhaust Particle exposure of epithelial cells causes release of GM-CSF which induces DC maturation and T cell proliferation (Bleck et al 2006)
• TLR4-dependent activation of structural epithelial cells (GM-CSF, TSLP, IL-25 and IL-33) in mice is necessary and sufficient for DC activation and priming of T cell to HDM (Hammad & Chieppa et al 2009)
The Epithelial Barrier in Asthma
Bronchial Asthma

- Inflammatory disease of the conducting airways which affects 1 in 7 children and 1 in 12 adults in UK
- Airways undergo distinct structural and functional changes leading to non-specific bronchoconstriction and airway obstruction
- Airways of asthmatics contract too much and too easily spontaneously and in response to environmental factors
- Asthma = Inflammation + non-specific bronchial hyperresponsiveness

Narrowing of Airways caused by
- Smooth muscle contraction
- Oedema / swelling
- Increased mucus secretion
- Inflammation

Fundamental feature of Asthma associated with allergic sensitisation is the ability of the airway to recognize common allergens and generate a Th2 type response
The Bronchial Wall In Asthma

Atopy and Asthma

- Fundamental feature of Asthma associated with allergic sensitisation is the ability of the airway to recognize common allergens and generate a Th2 type response

- 40% of western population is atopic (elevated IgE to common allergens) but only 7% express their atopy in form of asthma

- Non-atopic and atopic asthma have similar pathologies

- Need to understand specific expression of atopy in the conducting airways and understand how some patients despite being highly atopic have no evidence of asthma

- How is the immune response to allergens regulated at the surface of the airways?
Ag Sampling at the Epithelial Barrier

- In gut DCs sample allergens by increasing TJ proteins therefore can access lumen without compromising barrier function
- In Allergic Rhinitis, DCs express claudins implying DCs can sample Ag while keeping the epithelial barrier intact
- Does a similar process occur in the airways of asthmatics?
Interaction of allergens with the Epithelial Barrier

- Disruption of the epithelial barrier
- Release of mediators in response PAR activation
- Autoadjuvant properties

TLR4 on Structural cells induces Asthma in a mouse model

WT > WT = TLR4 on all cells
WT > TLR4KO = TLR4 on immune cells only
TLR4 KO > WT = TLR4 on structural cells only
TLR4 KO > TLR4 KO = absence of TLR4

Evidence of Damage at the Epithelial Barrier in Asthma

- Increasing severity of Asthma the greater the epithelial barrier is disrupted
- Increased EGFR expression with asthma severity
- Places the epithelium at the centre of Asthma pathogenesis

The Epithelial Barrier Is Disrupted in Asthma

Bronchial Biopsy

Differentiated Epithelial Cultures


Inherent Defect in Epithelial cultures in ionic and macromolecular permeability

TER

FITC-dextran

Environmental Factors Disrupt the Epithelial Barrier

Environmental stimuli include
- Air pollution, incl. cigarette smoke
- Allergens
- Occupational chemicals
- Viruses
- Bacterial products
- Fungal extracts

Host Stimuli
- Cytokines (IL-13, TNF-α)

Swindle et al (unpublished)
..and Results In Dendritic Cell Activation

1) HLA-DR

- **DC ONLY**
- **DC + 16HBE**

2) CD86

- **DC ONLY**
- **DC + 16HBE**

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Swindle et al (unpublished)
The Epithelial Mesenchymal Trophic Unit in Asthma - Inflammation in context!

Dendritic cell

Th-2 cell

IL-9, IL-5, GM-CSF

Mast cell

IL-3, IL-4

Eosinophil

Basophil

Th-2 cytokines

Environmental agents
Inflammatory cell products

Environmental agents
Inflammatory cell products

mucus

(myo) fibroblasts

Initiation

TGFβ

Amplification

VEGF

ET-1

Propagation

Smooth muscle

Blood vessels

The Epithelial Mesenchymal Trophic Unit

Chemoattractants, proinflammatory mediators

Davies et al JACI. 2003;111(2):245-255
Why is the barrier abnormal?

- Genetic factors (eg. DPP10, ORMDL3, GPR154, CHI3L1, SOCS1)
- Increased sensitivity to oxidative stress (genetic, dietary?)
- The cytokine milieu (eg. IL-13, TNF-α)
Interactions of Epithelial Cells and DCs in Asthma
The Epithelial barrier...

- is integral to the local control of tissue homeostasis
- is defective in asthma
- is sensitive to stimuli involved in exacerbations of asthma
- from asthmatic subjects is defective in response to stimuli involved in asthma exacerbations
- influences dendritic cell maturation with potential for involvement in development and maintenance of allergy
In vitro Models of the Epithelial Barrier
Bronchoscopy: A Wealth Of Patient Samples

- **BIOPSIES**
  - Explants
  - Smooth muscle cells (Myo)fibroblasts
  - Epithelial cells

- **BRUSHINGS**
  - Inflammatory cells
  - Airways lining fluid

- **BRONCHOALVEOLAR LAVAGE**
  - Leukocytes
  - Serum/plasma

- **BLOOD**
Primary Bronchial Epithelial Cell Culture

1) Epithelial cells isolated by brushing the airways of patients

2) Cells grown to confluence in laboratory using specialized medium (Px2)

3) Cells transferred to transwells and following overnight submerged culture taken to Air-Liquid Interface next day and cultured for further 21 days

2-3 wk

3-4 wk

Air

liquid
Models Of The Human Airway

- **Monocultures**: Individual cell types cultured ex vivo. Easy to work with. Obtain ALI cultures of BECs containing goblet cells and ciliated cells. Response of a single cell type over time. Cell-to-cell interactions cannot be investigated. Static cultures. Lacks complexity.

- **Human bronchial equivalents**: BECs and FBs coculture. Close interactions of two different cell types. All of BECs supported by underlying FBs. Technically challenging. Cell types separated by membrane support. Cells from different sources.

- **Tissue explant models**: BECs and FBs migrate out of bronchial biopsy and form two cell layers. Cells in correct orientation. Direct contact between FBs and BECs. Both cell types from same location in biopsy. Technically challenging. No circulation. Variability between replicates from same donor.

- **Models of the airway-blood barrier**: BECs and ECs (HMEC) coculture. Interaction of EC and BECs. Obtaining bronchial ECs.

- **Models of the airway incorporating immune cells**: BEC cultures with DCs and macrophages. Influence of BEC on immune cells. Ag sampling across epithelial barrier. Different media requirements for each cell type. Submerged cultures.

- **Tissue explant models**: Bronchial biopsy. All cells present in the airway are there in the correct orientation. Tissue direct from the airways. Short-term stimulation. Limited culture period. No circulation.

- **Lab-on-a-chip models of the airway**: BECs, ECs, FBs in microfabricated devices. Continuous monitoring of cell responses. Long-term cell culture. Apical delivery of stimulants. Monitor cell migration. Low cell numbers required. Technically challenging. Most models use cell lines.

**Abbreviations**: AI: Air-liquid interface; BEC: Bronchial epithelial cell; DC: Dendritic cell; EC: Endothelial cell; FB: Fibroblast; HMEC: Human umbilical vein endothelial cell.

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