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Epithelial barrier function: At the front line of asthma immunology and allergic airway inflammation

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Airway epithelial cells form a barrier to the outside world and are at the front line of mucosal immunity. Epithelial apical junctional complexes are multiprotein subunits that promote cell-cell adhesion and barrier integrity. Recent studies in the skin and gastrointestinal tract suggest that disruption of cell-cell junctions is required to initiate epithelial immune responses, but how this applies to mucosal immunity in the lung is not clear. Increasing evidence indicates that defective epithelial barrier function is a feature of airway inflammation in asthmatic patients. One challenge in this area is that barrier function and junctional integrity are difficult to study in the intact lung, but innovative approaches should provide new knowledge in this area in the near future. In this article we review the structure and function of epithelial apical junctional complexes, emphasizing how regulation of the epithelial barrier affects innate and adaptive immunity. We discuss why defective epithelial barrier function might be linked to T_H2 polarization in asthmatic patients and propose a rheostat model of barrier dysfunction that implicates the size of inhaled allergen particles as an important factor influencing adaptive immunity. (J Allergy Clin Immunol 2014;134:509-20.)

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Airway epithelial cells are an important part of the innate immune system in the lung. In addition to establishing mucociliary clearance, epithelial cells produce antimicrobial peptides, chemokines, and cytokines that recruit and activate other cell types and promote pathogen clearance.[1] Recent studies have emphasized the importance of epithelium-derived cytokines in promoting T_H2 immune responses, at least in part by conditioning local dendritic cells (DCs).[2,3] Epithelial cells also form a barrier to the outside world comprised of airway surface liquids, mucus, and apical junctional complexes (AJCs) that form between neighboring cells. AJCs consist of the apical ***tight junctions*** (TJs) and underlying ***adherens junctions*** (AJs) that bind together through homotypic and heterotypic interactions (Fig 1). Epithelial TJs and AJs establish cell-cell contact and cell polarity and also regulate the paracellular movement of ions and macromolecules. Recent studies have documented the presence of dysfunctional epithelial AJCs in the airways of asthmatic patients, although the precise mechanisms involved and consequences for airway inflammation are not clear. Interestingly, inhaled allergens, pollution particles, and respiratory tract viruses can disrupt barrier integrity, which might represent a risk factor for allergen sensitization. Certain inflammatory cytokines can also cause barrier dysfunction, potentially creating a positive feedback loop. In addition to allowing better penetration of inhaled allergens and particles, airway barrier dysfunction likely initiates signal transduction cascades, affecting epithelial activation and differentiation. Therefore regulation of airway epithelial barrier function is emerging as an important checkpoint in asthma immunology. Before considering the mechanisms and consequences of barrier dysfunction for allergic airway inflammation, a brief overview of junctional structure is in order.

Abbreviations used

AJ:	Adherens junction
AJC:	Apical junctional complex
ALI:	Air-liquid interface
CAR:	Coxsackie adenovirus receptor
CCSP:	Clara cell secretory product
DC:	Dendritic cell
HDM:	House dust mite
JAM:	Junctional adhesion molecule
MDCK:	Madin-Darby canine kidney
PBEC:	Primary bronchial epithelial cell
RSV:	Respiratory syncytial virus
TAMP:	Tight junction–associated MARVEL protein
TJ:	Tight junction
ZO:	Zonula occludens

AJCs: BASIC STRUCTURE AND FUNCTION

Junctions between neighboring cells were first discovered by using electron microscopy and appear as apposing strands that eliminate the intercellular space.[4] Junctional complexes contain

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Terms in boldface and italics are defined in the glossary on page 510.

the most apical TJs and underlying AJs, which are both linked to perijunctional ***actin*** filaments (Fig 1).[5,6] TJs regulate paracellular transport of ions and certain small molecules, whereas AJs are important for initiation and maintenance of cell-cell adhesion.[7,8] TJs and AJs interact to establish apical versus basolateral membrane domains (ie, cell polarity) and also regulate each other's structure. Both TJs and AJs are involved in numerous signal transduction cascades.[9] A current model proposes that there are 2 pathways for paracellular movement of molecules across TJs. The claudin-containing "pore" controls movement of ions in a charge and size-selective manner, whereas the "leak" pathway allows limited movement of larger macromolecules.[8] The precise molecular basis for size and charge discrimination by different junctional components is currently under active investigation, and several reviews are available on this topic.[8,10,11]

Our current understanding of junctional structure and function comes largely from studies of epithelial monolayers *in vitro*, and a current challenge is to understand how findings in model epithelia extrapolate to the multicellular epithelium in real-world conditions. *In vitro* studies typically grow epithelial monolayers to confluence on semipermeable membranes and compare epithelial barrier function with junctional structure, as determined by using microscopy. Different functional assays can be used to study barrier integrity. ***Transepithelial electrical resistance (TEER)*** is easy to measure and commonly used to assess junctional integrity because intact junctions will be relatively impermeable to ion flux (ie, high TEER). However, low TEER does not always imply higher macromolecular permeability (discussed in Rezaee et al[12]), and consequently, multiple approaches should be used to provide a complete picture of junctional integrity. In later sections of this review, we discuss additional assays that have been used to study outside-in airway barrier function in living organisms.

TJs and AJs are macromolecular complexes that bind together in the intercellular space and also make numerous intracytoplasmic protein-protein interactions. Table I summarizes the major families of junctional complex proteins, including the 3 TJ families. New junctional components and protein interactions are being discovered regularly, and Table I is meant to be illustrative rather than comprehensive.

First, claudins are a large family of tetraspanning transmembrane proteins that are expressed in a tissue- and cell type–selective manner and interact in a homotypic or heterotypic fashion in the extracellular space. Claudins can be either barrier promoting or barrier disrupting (or "leaky"). For example, claudin-1, the founding family member,[13] is necessary and sufficient for junction formation function.[14] Claudin-1–deficient mice die soon after birth and have excessive ***transepidermal water loss (TEWL)***.[15] This study established a key role for keratinocyte TJs in skin barrier function. Interestingly, defective expression of epidermal claudin-1 was observed in the skin of patients with atopic dermatitis,[16] where it can serve as a risk factor for viral infection and allergen sensitization.[17,18] Claudin-2, in contrast, is an example of a leaky claudin associated with increased permeability in the intestine, where it is induced by ***IL-13*** in a signal transducer and activator of transcription 6–dependent manner.[19] Although IL-4 and IL-13 also enhance airway epithelial permeability and barrier dysfunction, they do so without inducing claudin-2 in 16HBE airway epithelial cells.[20] These studies indicate that T_H2 cytokine–induced epithelial barrier dysfunction can occur in the intestine and airway through different mechanisms. Other claudins expressed in the respiratory tract include claudin-1, claudin-3, claudin-4, claudin-7, and claudin-18, the expression and function of which are under active study.[10-12,21,22]

The second group of TJ proteins is the tight junction–associated MARVEL protein (TAMP) family, which has 3 members: occludin, tricellulin, and MarvelD3.[23] In contrast to claudins, TAMP family members are not essential for normal epithelial development and barrier function, although they appear

GLOSSARY

ACTIN: A protein found especially in microfilaments and active in cellular movement and maintenance of cell shape. A belt of actin below the plasma membrane helps maintain the integrity of cellular junctions.

ADHERENS JUNCTION: These junctional structures form below tight junctions and help establish barrier function and epithelial polarity.

***Alternaria alternata*:** An aeroallergen of the Ascomycota phyla. Its spores have characteristic, elongated, beak-like chains. Spores are capable of traveling hundreds of miles and are found in grain-growing regions of temperate climates, with a peak in the late summer and fall. It is one of the most common spores found in dust from North American homes.

EPITHELIAL-MESENCHYMAL TRANSITION (EMT): A biologic process in which polarized epithelial cells assume a more mesenchymal phenotype characterized by migration and invasiveness. An early event in EMT is loss of junctional protein expression, including E-cadherin.

IL-13: A cytokine produced by T_H2 and type 2 innate lymphoid cells capable of inducing the IgE isotype switch. Its receptor is not found on mast cells (as is the case for IL-4), but IL-13 is more widely produced than IL-4. IL-13 contributes to airway mucus hypersecretion and airway hyperreactivity in mouse models.

PROTEASE-CONTAINING ALLERGENS: Cysteine and seine proteases are found in many common allergens, including fungal and insect extracts (eg, dust mite and cockroach). Allergen-associated proteases might promote allergic sensitization by disrupting epithelial junctional structures.

γδ T CELLS: A subset of T cells whose T-cell antigen receptors (TCRs) have γ and δ chains. These cells express a restricted repertoire of TCRs. They are capable of responding to nonpeptide and nonprocessed antigens, such as lipids, and appear to recognize antigens directly (independent of class I or class II MHC).

TIGHT JUNCTION: A multisubunit complex of transmembrane proteins that interact in the intercellular space to promote epithelial apposition. Tight junctions are comprised of different family members (eg, claudins and occludin) and link to the actin cytoskeleton.

TOLL-LIKE RECEPTOR 4 (TLR4): The first TLR identified. TLR4 binds to bacterial endotoxin (an LPS in the cell membrane of gram-negative bacteria) and viral coat proteins. Binding to TLR4 activates signal transduction through the MyD88 adaptor protein.

TRANSEPITHELIAL ELECTRICAL RESISTANCE (TEER): Opposition of the epithelium to the passage of a steady electrical current, which measures instantaneous ion flux. High TEER implies low ion flux and a tight epithelial barrier.

TRANSEPIDERMAL WATER LOSS (TEWL): A noninvasive measurement that uses vapor pressure gradient estimation. Humidity and temperature affect its measurement. TEWL is increased in patients with atopic dermatitis, reflecting defective skin barrier properties.

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