Phenotypic and genetic aspects of epithelial barrier function in asthmatic patients

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The bronchial epithelium is continuously exposed to a multitude of noxious challenges in inhaled air. Cellular contact with most damaging agents is reduced by the action of the mucociliary apparatus and by formation of a physical barrier that controls passage of ions and macromolecules. In conjunction with these defensive barrier functions, immunomodulatory cross-talk between the bronchial epithelium and tissue-resident immune cells controls the tissue microenvironment and barrier homeostasis. This is achieved by expression of an array of sensors that detect a wide variety of viral, bacterial, and nonmicrobial (toxins and irritants) agents, resulting in production of many different soluble and cell-surface molecules that signal to cells of the immune system. The ability of the bronchial epithelium to control the balance of inhibitory and activating signals is essential for orchestrating appropriate inflammatory and immune responses and for temporally modulating these responses to limit tissue injury and control the resolution of inflammation during tissue repair. In asthmatic patients abnormalities in many aspects of epithelial barrier function have been identified. We postulate that such abnormalities play a causal role in immune dysregulation in the airways by translating gene-environment interactions that underpin disease pathogenesis and exacerbation. (J Allergy Clin Immunol 2017;139:1736-51.)

Key words: *Asthma, tight junction, innate immunity, cytokine, homeostasis*

Abbreviations used

AhR:	Aryl hydrocarbon receptor
AJ:	Adherens junction
BHR:	Bronchial hyperresponsiveness
CDHR3:	Cadherin-related family member 3
DC:	Dendritic cell
DUOX1:	Dual oxidase 1
EGFR:	Epidermal growth factor receptor
eQTL:	Expression quantitative trait locus
GC:	Gene cluster
GST:	Glutathione-S-transferase
GWAS:	Genome-wide association study
ILC:	Innate lymphoid cell
ILC2:	Type 2 innate lymphoid cell
NK:	Natural killer
ORMDL3:	Orosomucoid-like 3
PCDH1:	Protocadherin 1
SC:	Subject cluster
SNP:	Single nucleotide polymorphism
TJ:	Tight junction
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin

ASTHMA HETEROGENEITY

Asthma is a common chronic inflammatory disorder of the conducting airways, which undergo distinct structural and functional changes leading to nonspecific bronchial hyperresponsiveness (BHR) and variable airflow obstruction. Recruitment and careful clinical characterization of large cohorts of asthmatic patients has established beyond doubt that asthma is a heterogeneous disease in terms of phenotype, endotype (ie, underlying pathogenic mechanism), response to treatment, and/or long-term clinical outcomes.[1] Cluster analysis has enabled identification of 4 to 5 phenotypic clusters that have differences in sex, asthma onset, lung function, atopic status, asthma control, health care use, and exacerbation frequency.[2-5] Molecular phenotyping of blood, induced sputum, and epithelial brushings has identified additional heterogeneity, especially in patients with severe asthma,[6-9] who are a major economic burden on the health care system because of poor responses to traditional asthma

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Terms in boldface and italics are defined in the glossary on page 1737.

medications. Some of the differences in asthma clusters might reflect underlying genetic differences; for example, there are differences in genetic risk in early-onset compared with ***late-onset asthma***,[10] whereas others might reflect differences in environment and lifestyle or, perhaps most likely, a combination of both gene and environment effects.[11] Many, but not all, asthmatic patients have T_H2 inflammation in their airways, and clinical trials with mAbs to IL-5, IL-13, or IL-4 receptor (α chain) have identified a type 2 endotype.[12] Thus patient stratification with type 2 relevant biomarkers has enabled effective targeting of these treatments to subsets of patients with moderate and severe asthma.[13-17] However, although clinical trials have shown that type 2 inflammation is an important disease modifier in some patients, they have also highlighted that non–type 2 inflammatory pathways must contribute to certain forms of asthma.[18] These can include pathways associated with obesity or neutrophilia or with susceptibility to environmental factors, such as infection and air pollution, but disease mechanisms/endotypes are not well understood. We postulate that a dynamic interaction between a genetically susceptible epithelium and environmental risk factors for asthma is important for the development of asthma and its subphenotypes.[19]

BRONCHIAL EPITHELIAL BARRIER STRUCTURE AND FUNCTION

Given the multitude of challenges imposed on the airway epithelium, it is not surprising that it combines structural and functional protective mechanisms with innate immunologic mechanisms to maintain healthy barrier homeostasis and to minimize inflammation and cellular dysregulation. Structurally, the bronchial epithelium is ***pseudostratified***, comprising mainly columnar multiciliated, secretory (goblet), and undifferentiated cells that overlie smaller basal cells with the capacity for self-renewal.[20] Rare cell types include pulmonary neuroendocrine cells[21,22] and brush (tuft) cells[23] that can have neurosensory or chemosensory functions, but information on these cells is limited.

On the epithelial surface, the mucociliary apparatus is a crucial primary innate defense mechanism that protects the lungs from deleterious effects of inhaled pollutants such as noxious gases and ***particulate matter,*** allergens, and pathogens. Surface epithelial cells and submucosal glands produce secretions comprising a superficial gel or mucus layer and a layer of periciliary fluid that contacts the epithelial surface. Mucus contains hydrated gel-forming mucins and a range of host defense and cytoprotective molecules, including ***defensins***, IgA, lactoperoxidase, catalase, superoxide dismutase, and low-molecular-weight antioxidants.[24] The viscoelastic properties of the mucus are dictated in large part by the oligomeric secreted mucins MUC5AC and MUC5B,[25] multifunctional glycoproteins that provide the structural framework of the mucus barrier. These bronchial secretions shield the epithelial surface, detoxify noxious agents, and trap many inhaled particles, allowing clearance through the action of the mucociliary escalator. MUC5B might also contribute to immune homeostasis by means of direct regulation of leukocyte functions.[26,27]

GLOSSARY

CIGARETTE SMOKE: Gases, hydrocarbon vapors, and particulate matter generated by burning tobacco. Cigarette smoke contains around 4000 substances, more than 60 of which have been identified as carcinogens. Cigarette smoke promotes increased local elastase production, which contributes to lung tissue injury likely caused by increased epithelial permeability through loss of tight junction integrity.

DEFENSINS: A distinct family of antimicrobial peptides produced by epithelial cells of mucosal surfaces, as well as by neutrophils, natural killer cells, and cytotoxic T lymphocytes. Defensins have direct antimicrobial activity, as well as the ability to activate inflammatory responses.

EPISTASIS: The expression of one gene is influenced by the expression of 1 or more independently inherited (nonallelic) genes.

GLUTATHIONE: An intracellular antioxidant whose principal site of synthesis is the liver. Glutathione serves as a cofactor for glutathione peroxidase, which is responsible for detoxifying lipid peroxides.

INNATE LYMPHOID CELLS: Cells possessing lymphoid morphology but without antigen receptors. Their subpopulations are divided into groups that resemble T helper subsets. Group 2 innate lymphoid cells produce type 2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, on stimulation with epithelium-derived cytokines, such as IL-33, IL-25, and TSLP.

LATE-ONSET ASTHMA: Often defined as asthma that develops in adolescence or adulthood. Most cases of asthma are diagnosed in childhood. Late-onset asthma tends to be more common in women than in men. Occupational asthma and aspirin-exacerbated respiratory disease are subtypes of late-onset asthma. Smoking and passive smoke exposure appear to be risk factors for late-onset asthma.

NOD-LIKE RECEPTORS (NLRs): A family of cytoplasmic receptors that recognize bacterial products, such as bacterial peptidoglycan. NLRs include an N-terminal effector region and a central nucleotide oligomerization domain (NOD), and most have C-terminal leucine-rich repeats for ligand (PAMP) binding. Once activated, NODs assemble signaling proteins, resulting in nuclear factor κB and mitogen-activated protein kinase activation, and control the activation of inflammatory caspases.

PARTICULATE MATTER (PM): PM is a term describing airborne dust particles originating from a range of sources and processes, such as fossil fuel combustion, waste incineration, cigarette smoking, and erosion, which can contain black carbon (soot), metals, polyaromatic hydrocarbons, and anions, such as sulfate and nitrate, among others. If sufficiently small to be inhaled (aerodynamic diameter <10 μm), PM can settle in the airways and exert a range of effects.

PATHOGEN-ASSOCIATED MOLECULAR PATTERN (PAMP): Molecules associated with groups of pathogens that are recognized as "danger signals" by pattern recognition receptors, such as Toll-like receptors or NOD-like receptors. Many types of molecules can serve as PAMPs, including LPSs, peptidoglycans, lipoteichoic acid, nucleic acid variants, and flagellin.

POLYAROMATIC HYDROCARBONS (PAHs): Also known as polycyclic aromatic hydrocarbons, PAHs are volatile substances produced by cooking oils and coal-burning or petroleum products. Indoor biomass burning generates PAHs and is associated with chronic obstructive pulmonary disease development in women.

PSEUDOSTRATIFIED: Although showing features of layering in an epithelium, all cells are still attached to the basement membrane.

TOLL-LIKE RECEPTORS (TLRs): TLRs are a family of innate pattern recognition receptors that respond to a variety of structurally conserved molecules derived from pathogens (ie, PAMPs). They are single, membrane-spanning, noncatalytic receptors whose signaling pathways are finely regulated by Toll/IL-1 receptor homologous region (TIR) domain–containing adaptors. Differential use of these adaptor proteins provides specificity of individual TLR-mediated signaling pathways.

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