Etiology of epithelial barrier dysfunction in patients with type 2 inflammatory diseases

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Epithelial barriers of the skin, gastrointestinal tract, and airway serve common critical functions, such as maintaining a physical barrier against environmental insults and allergens and providing a tissue interface balancing the communication between the internal and external environments. We now understand that in patients with allergic disease, regardless of tissue location, the homeostatic balance of the epithelial barrier is skewed toward loss of differentiation, reduced junctional integrity, and impaired innate defense. Importantly, epithelial dysfunction characterized by these traits appears to pre-date atopy and development of allergic disease. Despite our growing appreciation of the centrality of barrier dysfunction in initiation of allergic disease, many important questions remain to be answered regarding mechanisms disrupting normal barrier function. Although our external environment (proteases, allergens, and injury) is classically thought of as a principal contributor to barrier disruption associated with allergic sensitization, there is a need to better understand contributions of the internal environment (hormones, diet, and circadian clock). Systemic drivers of disease, such as alterations of the endocrine system, metabolism, and aberrant control of developmental signaling, are emerging as new players in driving epithelial dysfunction and allergic predisposition at various barrier sites. Identifying such central mediators of epithelial dysfunction using both systems biology tools and causality-driven laboratory experimentation will be essential in building new strategic interventions to prevent or reverse the process of barrier loss in allergic patients. (J Allergy Clin Immunol 2017;139:1752-61.)

Key words: *Epithelial barrier, differentiation, mesenchyme, extracellular matrix, tight junctions, hormones, metabolism, allergic predisposition, allergic sensitization*

Abbreviations used
AD: Atopic dermatitis
CRS: Chronic rhinosinusitis
EDC: Epidermal differentiation complex
EMT: Epithelial-to-mesenchymal transition
EoE: Eosinophilic esophagitis
FLG: Filaggrin
ZO-1: Zonula occludens 1

The epithelial barrier is diverse, occurring *inter alia* in the gastrointestinal tract, urogenital tract, respiratory system, eyes, and skin. Epithelial cells are specialized in each of these organ systems and in the various geographic regions of each system. The main functions of epithelia are to present a physical and immune barrier; maintain a surface that accommodates commensal organisms but not pathogens; remove, degrade, or neutralize environmental toxins and particulates; and maintain the balance of water and electrolytes. Specialized functions of the physical barrier include maintenance of the mucociliary escalator in the airways, production of surfactants in the alveoli and small airways, and production of a protective mucus blanket in the gastrointestinal and urogenital tracts.

The phrase immune barrier refers to epithelial elements that are essential for innate immune resistance to potential pathogens, including constitutive and inducible expression of enzymes, peptides, proteins, lipids, ions, and pathogen recognition receptor systems designed to protect the host. Combined with epithelial tight junctions, these systems present a vigilant and effective barrier to microbial invasion. Many allergic diseases are now known to manifest clear disorders of the immune barrier. The purpose of this review is to discuss the presence of barrier dysfunction, its role in allergic disease, and the molecular and endocrinologic mechanisms that are underlying causes.

BARRIER DEFECTS IN PATIENTS WITH TYPE 2 DISEASES

Introduction

When the immune barrier is predisposed to disruption, microorganisms and antigens can gain access between epithelial cells through the basement membrane to the underlying lamina

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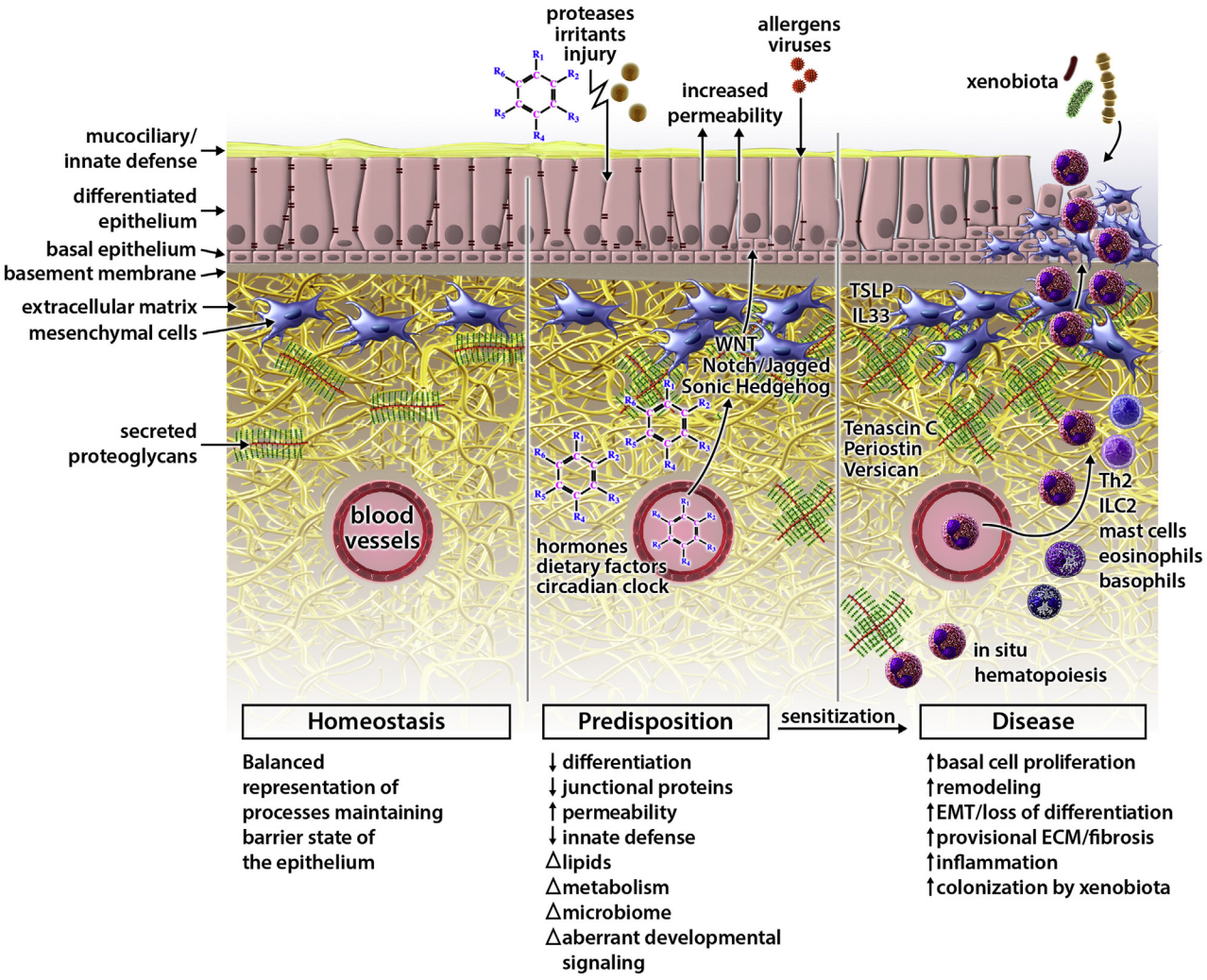


FIG 1. Key features of epithelial dysfunction common to all allergic disease. Epithelial barriers predisposed to type 2 allergic disease are characterized by increased permeability and aberrant behavior of morphogenetic programs that maintain epithelial homeostasis. Both exogenous (inhaled allergens, respiratory viruses, chemical sensitizers, and air pollutants) and endogenous (hormones, dietary factors, and altered circadian clock) disruptors of epithelial homeostasis can drive predisposition to allergic sensitization by altering homeostatic activity of developmental pathways (WNT, Notch, and Hedgehog) that maintain proper epithelial-to-mesenchymal communication, epithelial differentiation, and barrier integrity. Transition to a remodeling state in disease typically follows allergic sensitization and inflammatory responses. This transition features further loss of differentiation signals, downregulation of innate defense molecules, profibrotic processes, deposition of extracellular matrix, and potentiated activity of the mesenchymal unit. *ILC2*, Group 2 innate lymphoid cell; *TSLP*, thymic stromal lymphopoietin.

propria and connective tissue. Penetration of microbes triggers strong innate immune responses by pathogen recognition receptors on epithelial cells and immune cells, such as macrophages, innate lymphoid cells, and mast cells, residing among epithelial cells or on the basolateral side of the lamina reticularis. Subsequent sensitization and activation of adaptive immune responses can result, including type 2 immune responses of relevance to the allergic diathesis. Epithelial function can become compromised by alterations in numerous key functional or structural elements, such as epithelial junctional or filamentous proteins. Loss of protective anti-proteases, antimicrobial peptides or compounds, or disturbances in transport of ions, protons or water can also disrupt barrier function. Profound activation of sensory nerves important in manifestation of disease experienced by the patient is also associated with loss of barrier and sensitization. In this section we briefly discuss some of the salient mechanisms and features of barrier loss commonly shared by all patients with allergic diseases (summarized in Fig 1).

Atopic dermatitis

Atopic dermatitis (AD) is characterized by loss of barrier function of the skin, culminating in a clinical phenotype characterized by formation of skin lesions. Skin barrier dysfunction is induced by disruption of the stratum corneum, a dense protein-lipid matrix that functions as a barrier to water loss, environmental insults and allergens. Filaggrin (FLG), a filament-associated epidermal differentiation complex (EDC) protein essential for regulation of epidermal homeostasis, is highly deficient in the skin of many patients with AD.[1] Similarly, the FLG-like proteins hornerin and FLG2 are detected at

significantly lower levels in the skin of many patients with AD, irrespective of FLG genotype.[2]

Dysregulated FLG is frequently discussed as central to the origins of disease[3]; however, barrier defects in patients with AD go far beyond FLG deficiency. Defects in claudins 1, 4, and 8 have also been associated with the development of AD.[4-7] Disruption of the skin barrier involves defects in the entire keratinocyte terminal differentiation program through dysregulation of multiple EDC genes on human chromosome region 1q21,[8] reduced expression of epithelial tight junction proteins,[9] increased transepidermal water loss,[10,11] and reduction in epidermal natural moisturizing factors.[12] The impaired skin barrier function in patients with AD also involves altered lipid profiles, including shortening of carbon chain length of stratum corneum lipids (ceramides and free fatty acids), an event that exhibits strong correlations with transepidermal water loss and occurs independently of FLG mutations.[13-16] Intriguingly, clinically unaffected nonlesional skin in patients with AD similarly exhibits defects in terminal keratinocyte differentiation,[17] as well as reduced FLG and lipid levels,[14,15] suggesting a global cutaneous predisposition to barrier dysfunction in patients with AD.

Asthma

An intact functional mucosal barrier is crucial for maintenance of airway homeostasis. Despite the existence of multiple clinical endotypes, patients with most forms of asthma exhibit a dysregulated epithelial barrier. Asthmatic epithelium is characterized by an increase in basal and goblet cell counts[18] and a decrease in terminally differentiated ciliated cell counts that are frequently accompanied by basement membrane thickening[19,20] and epithelial shedding, with the formation of Creola bodies consisting of clusters of shed epithelium (even in patients with mild forms of the disease).[21-23] Disruption of epithelial tight and adherens junctions is typical for asthma, with marked loss of E-cadherin[24] and claudin-18.[25] The underlying extracellular matrix, which supports homeostasis and repair of the epithelium, also undergoes significant remodeling, which is characterized by increased deposition of provisional matrix components, such as the glycoproteins fibronectin,[26] periostin,[27] tenascin-C,[28,29] hyaluronan, and versican.[30] Epithelial-to-mesenchymal transition (EMT) was proposed as a mechanism underlying epithelial dedifferentiation and perpetuated remodeling[31]; however, this remains a topic of debate because of the lack of markers accurately defining this process in asthmatic patients.[32] Mucociliary clearance, which depends on cooperation between submucosal glands, goblet cells, and ciliated cells, and normal chemical barrier function of the asthmatic epithelium are also compromised. There are profound changes in relative proportions and viscosity of mucins MUC5AC and MUC5B, which in turn contribute to airway obstruction[33,34] and downregulation of lipoxins.[35] Perturbations in sphingolipid balance with increased levels of ceramides have also been reported in airway epithelium of asthmatic patients.[36]

Allergic rhinitis

Mucosal epithelial barrier disruption is observed in both models of allergic rhinitis and in patients.[37] Allergens can contain proteases and have been shown to disrupt epithelial tight junctions.[38,39] Nasal challenge with histamine or antigen in a sensitized subject can cause significant plasma exudation across the epithelial barrier. This is a nondestructive process and might be a first-line defense of the respiratory mucosa.[40] A proteomic study detected changes reflecting altered epithelial permeability in patients with allergic rhinitis, including increased α_2-macroglobulin levels, a measure of vascular and epithelial leak, and decreased levels of the protective antileukoproteinase secretory leukocyte peptidase inhibitor.[41] Reduced levels of the barrier proteins E-cadherin and zonula occludens 1 (ZO-1) were observed in nasal mucosal tissue from patients with allergic rhinitis compared with those in healthy control subjects by using real-time PCR, Western blotting, and immunohistochemistry.[42] *In vitro* IL-4 and TNF induced loss of these markers in the epithelium. Steelant et al[43] observed decreased barrier function measured *in vitro* by using primary epithelial cells collected in patients with allergic rhinitis compared with healthy control subjects and found decreased expression of the tight junction proteins occludin and ZO-1.

Chronic rhinosinusitis

Early studies provided evidence for a defective barrier in patients with chronic rhinosinusitis (CRS) by using immunohistochemistry, bioelectric evaluation, and ion transport function as indicators.[44,45] Using air-liquid interface epithelial cultures, Soyka et al[46] showed reduced barrier function in tissues collected from patients with or without CRS. A number of studies have focused on loss of epithelial tight junctions in patients with CRS by assessing levels of individual tight junction proteins.[37,47] Alterations of the expression of ZO-1 and E-cadherin were reported by Jang et al.[48] Epithelial damage, including shortening of desmosomes, was reported by Shahana et al[49] in a study using electron microscopy in polyp tissue derived from asthmatic patients. Reductions in levels of the tight junction proteins claudin-1 and occludin in tissues from patients with CRS were reported in another study.[50] Meng et al[51] reported that ZO-1, occludin, and E-cadherin levels were all reduced in mature polyps removed from patients with CRS with nasal polyps. Moreover, aquaporin 5, a marker of epithelial differentiation, was significantly reduced in sinonasal samples of patients with CRS with nasal polyps compared with levels in those with CRS without nasal polyps or control subjects.[52] Physical evidence of disruption of epithelial barrier in patients with CRS includes prominent acanthosis and acantholysis.[47] The loss of differentiation and acanthosis might result from a cycle of ongoing injury and repair associated with EMT. Factors that have been identified in tissues from patients with CRS that might stimulate barrier loss and acanthosis include TGF-α, oncostatin M, epiregulin, hypoxic activation of hypoxia-inducible factor 1α, and endocrine deficiency (see below).[47,53-55]

Eosinophilic esophagitis

The esophagus is composed of stratified squamous epithelium remarkably similar to the epithelium of the skin, only differing in its lack of a cornified layer and its possession of a mucous layer.[56] As occurs in patients with other type 2 diseases, the normal structure of the epithelium is disrupted in patients with eosinophilic esophagitis (EoE), including basal cell hyperplasia, dilated intracellular spaces, and impaired barrier function and

cell junctions.[57,58] These changes are thought to be associated with dysregulated epithelial differentiation and impaired epithelial barrier formation. As in the skin, the epithelium in patients with EoE is characterized by dysregulation of genes of the EDC on human chromosome 1q21, including *FLG*, involucrin, and several small proline-rich repeat family members.[59] In esophageal epithelial cells, FLG was shown to be negatively regulated in response to IL-13 *ex vivo*.[59] Levels of the desmosomal and tight junctional proteins desmoglein-1, E-cadherin, and claudin-1 are reduced in patients with active EoE.[60,61] Epithelial barriers in patients with EoE also exhibit characteristic features of EMT, which correlate with subepithelial fibrosis and eosinophil counts in human biopsy specimens.[62]

MECHANISMS OF BARRIER DEFECTS

Introduction

Barrier defects can be induced by loss or defects in key proteins that comprise tight or adherens junctions, disruption of barrier by environmental exposures (including proteases, chemical injury or trauma), inflammatory responses that induce barrier-disrupting T_H2 cytokines, and endogenous mechanisms, such as altered central metabolism and imbalance of hormones that regulate epithelial homeostasis. The purpose of this section is to briefly consider some of the external and internal factors thought to be of particular importance in disruption of barriers in patients with allergic diseases.

Genetic and epigenetic barrier defects

Genome-wide association studies and positional cloning have successfully identified several risk alleles and loci reproducibly associated with AD, asthma, and EoE. Interestingly, many allergic susceptibility candidate genes control epithelial barrier homeostasis. Null gene mutations of *FLG* (as mentioned previously, an important component of terminal keratinocyte differentiation) are the most significant known risk factors for AD and EoE, along with mutations of other EDC genes.[59] Polymorphisms in the asthma susceptibility genes *ORMDL3*, *GSDMB*, *PCDH1*, *CDHR3*, *ADAM33*, *SMAD3*, *IL1RL1*, and *IL18R1* are now thought to be linked to aberrant epithelial remodeling, the unfolded protein response, and lipid biosynthesis.[63-65] A recent pathway-based association study by Barreto-Luis et al[66] revealed novel asthma susceptibility loci near WNT pathway genes that regulate barrier morphogenesis.

Even though genetic associations are stronger within clinically well-defined subgroups of disease,[67] overall allergic disease–associated alleles typically have small effect sizes and cannot account for the rapidly increasing prevalence of allergy,[68] reinforcing the significance of environmental factors in development of allergic diseases. Significantly, environmental inputs can affect gene transcription through heritable epigenetic regulation that does not require alterations in gene sequences, including DNA and histone modifications, as well as changes in noncoding RNAs.[69] One of the notable epigenetic changes in asthmatic epithelium involves hypomethylation of KRT5,[69] which increases keratin 5 expression in basal epithelium and can therefore lead to dysregulated epithelial differentiation.[70-72] The potential for epigenetic "reprogramming" is evident when asthmatic epithelium cultured in normal medium still retains persistent indicators of defects in junctional maintenance, "immaturity," and repair *ex vivo*.[73] The topic of epigenetic mechanisms of epithelial remodeling is a still-emerging area of investigation of epithelial barrier dysfunction, and ongoing work is likely to produce novel insights into aberrant regulation of epithelial differentiation programs in patients with allergic disease.

Environmental contributions to barrier loss

Barriers of the skin, airways, and gastrointestinal tract constantly experience biological and chemical insults from the surrounding environment. Allergens, such as house dust mite or pollens, are capable of disrupting the physical integrity of the barrier through their protease activity, which degrades adhesion proteins and triggers the epithelial alarmin cytokine response.[74-78] Respiratory viruses promote airway epithelial barrier dysfunction by disrupting epithelial junctions, which might represent an inciting or sustaining event linking viral infections and allergic inflammation.[79,80] Interestingly, the asthma susceptibility barrier gene *CDHR3* is a receptor for rhinovirus C.[65] Many chemicals and irritants prevalent in industrial environments act as adjuvants and sensitizers, disrupting normal functions of the epithelial barrier, setting off alarmin responses, and promoting allergic sensitization.[81,82] Air pollution associates with the development of AD in both children and adults.[83,84]

Less obvious but equally important environmental contributors to barrier loss do not disrupt barrier externally but act as endogenous factors in dysregulation of epithelial homeostatic processes. Stimulation of the aryl hydrocarbon receptor by ingested or inhaled xenobiotics, such as polycyclic aromatic hydrocarbons, is now known to affect epidermal differentiation and skin barrier formation.[85] Xenoestrogens linked to development of allergy, such as bisphenols and phthalates, mimic the natural action of nuclear hormonal receptor ligands (estrogens and androgens) and thus disrupt normal epithelial homeostatic processes.[86-88]

A multitude of dietary factors have the capacity to alter epithelial behavior through direct receptor action or indirect regulation of tissue metabolism. For example, Fischer et al[89] demonstrated that vitamin D supplementation, acting through the vitamin D receptor pathway, reduces EMT processes and improves barrier function in several clinically relevant murine models of asthma. Fatty acid–deficient diets spontaneously induce skin barrier disruption through alteration of skin metabolism.[90,91] Moreover, the intestinal microbiome is now emerging as an important regulator of the metabolism with consequences for epithelial and immune homeostasis.[92]

Defects in hormonal signaling as early events

Contributions of biological systems other than the immune system to disruption of epithelial homeostasis and priming of allergic response are currently poorly understood. Several studies report insulin resistance in children and adults with asthma,[93,94] as well as association of asthma and atopic eczema with prediabetes and metabolic syndrome.[95] We found profound changes in serum hormonal profiles of prepubertal nonobese allergic children compared with nonallergic control subjects, as well as patients with CRS compared with healthy control

subjects, including decreased levels of serum insulin and increased output of thyroid and growth hormones.[96] Hormones play a critical role in the maintenance of epithelial barrier homeostasis and integrity through their integration with epithelial morphogenetic programs,[88,97] as evidenced by deficiencies in wound healing and epithelial dysfunction in patients with thyroid disease and diabetes.[98,99] Insulin and insulin-like growth factor 1 are essential drivers of epithelial differentiation and regulators of energy metabolism. Double-transgenic mice lacking both insulin and insulin-like growth factor 1 receptors are characterized by severe defects in epithelial differentiation, severely impaired stratification of the epidermis, spontaneous lesions, and overall loss of skin barrier function.[100] Recently, sex steroids and estrogen in particular are receiving more attention for their potential to explain sex bias in the prevalence of allergic disease.[101] Glucocorticoids have profound effects on epithelial differentiation[102,103] and exemplify the therapeutic potential of manipulating the endocrine system in the treatment of allergic disease.

Disruption of normal epithelial development

The significance of early-life events in initiation and propagation of allergic disease is now widely recognized. The first 2 years of life represent a window of susceptibility for asthma development. Importantly, epithelial barriers in childhood are shaped and regulated by active ongoing developmental programs. In particular, morphogenesis of the lung epithelial barrier continues throughout normal postnatal development, as characterized by ongoing septation and epithelialization of the lung.[104] Consequently, perturbation of barrier morphogenesis in childhood can have a lasting effect on adult epithelium through alteration of developmental programs and epigenetic reprogramming at developmental checkpoints.[105-107] Multiple lines of evidence now implicate active re-engagement of morphogenetic programs in adult disease typically not seen in adult homeostatic tissue.[108-110] Alterations of the Wnt,[66] Hippo,[111] Notch/Jagged,[112,113] and Hedgehog[114] developmental pathways all exhibit strong association with epithelial remodeling and allergy.

Early disruption of normal barrier development frequently predates atopy[11,44] and is emerging as central to initiation of the atopic march. Mouse models of the atopic march demonstrate conclusively that sensitization to allergens through disrupted skin barriers is sufficient to elicit an immune response at other barrier sites.[115,116] Causes of disruption of epithelial morphogenesis early in life are not well understood. Early-life viral exposures[117,118] and changes in microbiota[119] are some of the better understood factors driving aberrant epithelial responses and a predisposition to allergic sensitization.

The injury-repair cycle and EMT

Exposure to environmental factors causes injury or disruption of normal homeostasis of the epithelium, followed by engagement of robust repair processes to minimize further damage from microbes and environment. Mature differentiated epithelial cells undergo EMT in which the cells lose their attachments to basement membrane and each other, lose their polarity, begin to divide, and become migratory to rapidly cover the injured area. During this process, epithelial cells lose

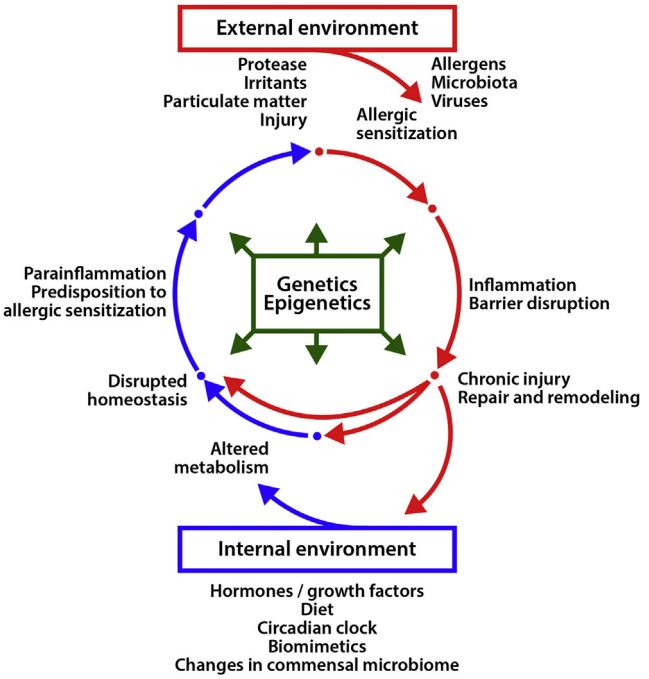


FIG 2. Changing states of the epithelial barrier on a homeostasis–allergic disease continuum. Dysregulation of normal epithelial barrier function is a gradual process involving multiple causal factors. This includes genetics, epigenetics, and influences of the external and internal epithelial environments.

expression of the tight junction proteins ZO-1, occludin, E-cadherin, and other markers of mature differentiated epithelium. The mesenchymal cells derived from the basal epithelial cells begin to produce desmin, fibronectin, tenascin, laminin, collagens, and other proteins that form a provisional matrix to protect the exposed basement membrane or lamina propria and express differentiation markers, such as α-smooth muscle actin. Evidence for EMT usually consists of loss of tight junction proteins, gain of provisional matrix proteins or biomarkers, or both. Early studies by Davies, Holgate, Hackett, Knight, and others found evidence for persistence of EMT in asthmatic patients,[120-123] although it is more often described as a mild "partial EMT" phenotype, which could contribute to poor expression of conventional EMT markers in airways of asthmatic patients.[32] More recent studies found similar evidence for increased EMT in patients with allergic rhinitis.[42,43] Several studies have demonstrated ongoing EMT in patients with CRS.[37,47,49-51] Two studies have demonstrated ongoing EMT in patients with EoE.[62,124] However, to date, the authors have not found any reports of EMT (as classically defined by developmental biologists) in patients with AD, despite abundant evidence for loss of epithelial differentiation programs in the skin of patients with AD. Thus most chronic type 2 inflammatory diseases often appear to have a chronic EMT-based ongoing injury-repair cycle.

Inflammation and barrier: A vicious cycle

Whether inflammation is the primary cause of barrier disruption or whether it is barrier dysfunction that leads to sensitization and aberrant inflammatory response poses a

long-standing "chicken-egg" dilemma (Fig 2). Aberrant innate immune activity at barrier sites favors sensitization to innocuous antigens, and if there is an ensuing adaptive immune response, it can lead to a full-scale inflammatory attack on epithelial cells. During chronic inflammation associated with repeated or persistent antigen exposure, inflammation can lead to persistent disruption of epithelial junctions, as well as epithelial remodeling and subepithelial fibrosis driven by chronic perpetuated repair. Conversely, an accumulating body of evidence points to a causal role for barrier dysfunction as a primary driver of the allergic response and a vicious cycle of barrier leak.[125-127] Strikingly, only 30% to 40% of cases of allergic disease (asthma, eczema, and allergic rhinitis) in early childhood are attributable to atopy, and 60% to 70% of cases result from yet unknown factors.[128] In most atopic march cases, increased skin permeability at birth and nonatopic eczema frequently predate development of atopy,[11,127] regardless of *FLG* mutations.[10] Moreover, although the atopic march assumes that one allergic disease brings risk of acquisition of another allergic disease later in life, it might involve sensitization to antigens different from those that triggered the initial disease.[129,130] From this perspective, an alternative view might be that atopy and allergic inflammation are both secondary to a yet-unknown systemic process progressively afflicting epithelial barriers of the skin, gut, and airway.

Based on our observations of hormonal imbalance in allergic children (described above), we hypothesize that dysregulation of the endocrine system could represent a missing systemic link underlying susceptibility to initial barrier dysfunction. Remarkable similarities in the biology of barrier dysfunction across different allergic diseases (Fig 1), including upper and lower "unified" airway responses and nonlesional defects in clinically unaffected skin of patients with AD, all support the existence of such a systemic trigger of disease.[131-133]

Transgenic mouse models with defects in proteins maintaining epithelial homeostasis, such as FLG, are also characterized by spontaneous allergic sensitization and enhanced allergic inflammation.[134-137] This notion of causality of a pre-existing barrier problem fits well with an emerging view of type 2 immunity as a "restorative" response evolved to maintain tissue homeostasis and assist with repair and remodeling.[138,139] For example, Huang et al[140] provide supporting evidence suggesting that T_H2 immune responses and homeostatic eosinophil activity[141] might have evolved not to expel parasites but rather to remodel tissue that contains parasitic infestation and minimize potential damage to the host.

IDENTIFYING KEY DRIVERS OF DEFECTS AND THE BASIS FOR PREDISPOSITION TO BARRIER DISEASE

The existence of a dilemma about whether it is barrier loss or sensitization that is the primary cause of this vicious cycle illustrates well the complexity of allergic pathogenesis (Fig 2). In healthy subjects multiple biological systems and processes must work in unison to maintain tissue homeostasis at barrier sites. This includes morphogenetic/developmental programs, metabolism, and the endocrine and immune systems. There is a growing list of molecules that we now believe are of critical importance in regulating barrier biology; however, despite exhibiting strong phenotypes in models of disease, they might not be central drivers of disease pathogenesis. FLG, for example, is only one of many molecules in the EDC.[142] Knockdown of FLG alone was not sufficient to affect lipid composition and permeability in an *ex vivo* skin model.[143] Several other EDC molecules play an equally critical role in epithelial homeostasis and are frequently downregulated with FLG in patients with AD independent of FLG genotype or atopic status.[8] Such changes in differentiation status of cells are intricately intertwined with changes in tissue metabolic demands,[144] aberrant immune responses,[145] and epigenetic rewiring.[146] The question then becomes which nongenetic factors have the capacity to dysregulate the entire EDC cluster, affecting the balance of the whole system. Identifying such central mediators is the key to finding strategies to restore the homeostatic state of the epithelial barrier. The endocrine system and changes in central metabolism have the potential to be central drivers of the predisposition to barrier defects, given the systemic regulatory effects of hormones (insulin and insulin-like growth factor 1 [IGF-1]) on epithelial morphogenetic programs of all barrier sites[147-149] and their regulatory roles for metabolism and immunity. Our understanding of central mediators of allergic disease is currently impeded by use of reductionist approaches and limited focus on processes of the immune system, insufficient understanding of complexity of biological interactions that control normal epithelial homeostasis, the dynamic nature of biological systems, and the extreme clinical heterogeneity of asthma and other allergic diseases. Studies of systems and integrative biology address exactly these challenges and are now emerging as critical in advancing all branches of biomedical science. Next-generation sequencing technology, shared availability of "big data," and advances in computational biology are new promising tools for the discovery of novel drivers of barrier dysfunction.[150,151]

TARGETS FOR THERAPEUTIC INTERVENTION

There are severe forms of the diseases discussed in this review (ie, asthma, allergic rhinitis, CRS, EoE, and AD) that are difficult to manage and for which there is a serious unmet medical need for new and more effective medications. At present, one of the most highly effective classes of drugs for these diseases is topical glucocorticosteroids. These drugs improve elements of innate immunity, as well as barrier function, as measured based on a number of criteria, including markers of tight junction presence and function. Although the benefit of corticosteroids derives from suppressing the inflammatory response, such as expression of IL-13 and other cytokines, as well as direct effects on epithelial cells to promote barrier integrity, the relative contribution of these effects is not clear. New classes of drugs are rapidly emerging to help manage disease in patients for whom glucocorticosteroids are inadequate to control disease. This includes small-molecule drugs that block signaling pathways (eg, chemoattractant receptor–homologous molecule [CRTH2] expressed on T_H2 lymphocytes, GATA-3, Janus kinase, and other kinases) and mAbs that block cytokine signaling or eliminate key allergic effector cells, such as eosinophils and mast cells (eg, IL-5, IL-13, IL-5 receptor α, IL-4/IL-13 receptor α, and sialic acid–binding immunoglobulin-like lectin 8). The pursuit of barrier restorative therapies is another emerging field.[152] Based on the hypothesis advanced here that endocrine defects are important in disease pathogenesis, it will be worthwhile to test manipulation of signaling of other hormones (eg, insulin, growth hormone, and thyroid hormone) when seeking benefit for patients.

SUMMARY AND CONCLUSIONS

Many important questions remain to be answered regarding the mechanisms by which the epithelial barrier becomes disrupted in patients with allergic diseases. We also need to better understand the role that barrier loss plays as both an initiator and a consequence of the inflammatory process. We need to know more about genetic and epigenetic factors that cause barrier loss, as well as the environmental triggers and cellular and molecular mediators and targets of the process. Identifying the central mediators of the response and the consequences of barrier dysfunction in disease will be essential in building a rationale and in strategic design of new interventions to prevent or reverse the process.

REFERENCES

1. Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol 2014;134:792-9.
2. Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Mechin MC, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol 2013;131:1094-102.
3. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006;38:441-6.
4. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol 2011;127:773-86, e1-7.
5. Tokumasu R, Yamaga K, Yamazaki Y, Murota H, Suzuki K, Tamura A, et al. Dose-dependent role of claudin-1 in vivo in orchestrating features of atopic dermatitis. Proc Natl Acad Sci U S A 2016;113:E4061-8.
6. Esaki H, Ewald DA, Ungar B, Rozenblit M, Zheng X, Xu H, et al. Identification of novel immune and barrier genes in atopic dermatitis by means of laser capture microdissection. J Allergy Clin Immunol 2015;135:153-63.
7. Yu HS, Kang MJ, Kwon JW, Lee SY, Lee E, Yang SI, et al. Claudin-1 polymorphism modifies the effect of mold exposure on the development of atopic dermatitis and production of IgE. J Allergy Clin Immunol 2015;135:827-30.e5.
8. Guttman-Yassky E, Suarez-Farinas M, Chiricozzi A, Nograles KE, Shemer A, Fuentes-Duculan J, et al. Broad defects in epidermal cornification in atopic dermatitis identified through genomic analysis. J Allergy Clin Immunol 2009;124:1235-44.e58.
9. Yuki T, Tobiishi M, Kusaka-Kikushima A, Ota Y, Tokura Y. Impaired tight junctions in atopic dermatitis skin and in a skin-equivalent model treated with interleukin-17. PLoS One 2016;11:e0161759.
10. Horimukai K, Morita K, Narita M, Kondo M, Kabashima S, Inoue E, et al. Transepidermal water loss measurement during infancy can predict the subsequent development of atopic dermatitis regardless of filaggrin mutations. Allergol Int 2016;65:103-8.
11. Kelleher M, Dunn-Galvin A, Hourihane JO, Murray D, Campbell LE, McLean WH, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. J Allergy Clin Immunol 2015;135:930-5.e1.
12. Chittock J, Cooke A, Lavender T, Brown K, Wigley A, Victor S, et al. Development of stratum corneum chymotrypsin-like protease activity and natural moisturizing factors from birth to 4 weeks of age compared with adults. Br J Dermatol 2016;175:713-20.
13. Janssens M, van Smeden J, Gooris GS, Bras W, Portale G, Caspers PJ, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res 2012;53:2755-66.
14. Joo KM, Hwang JH, Bae S, Nahm DH, Park HS, Ye YM, et al. Relationship of ceramide-, and free fatty acid-cholesterol ratios in the stratum corneum with skin barrier function of normal, atopic dermatitis lesional and non-lesional skins. J Dermatol Sci 2015;77:71-4.
15. van Smeden J, Janssens M, Kaye EC, Caspers PJ, Lavrijsen AP, Vreeken RJ, et al. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. Exp Dermatol 2014;23:45-52.
16. Li S, Villarreal M, Stewart S, Choi J, Indra G, Babineau DC, et al. Altered composition of epidermal lipids correlates with *Staphylococcus aureus* colonization status in atopic dermatitis. Br J Dermatol 2017 [Epub ahead of print].
17. Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol 2011;127:954-64, e1-4.
18. Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. Am J Respir Crit Care Med 2001;163:517-23.
19. Honkova L, Uhlik J, Berankova K, Svobodova T, Pohunek P. Epithelial basement membrane thickening is related to TGF-Beta 1 expression in children with chronic respiratory diseases. Pediatr Allergy Immunol 2014;25:593-9.
20. Zhou C, Yin G, Liu J, Liu X, Zhao S. Epithelial apoptosis and loss in airways of children with asthma. J Asthma 2011;48:358-65.
21. Trautmann A, Kruger K, Akdis M, Muller-Wening D, Akkaya A, Brocker EB, et al. Apoptosis and loss of adhesion of bronchial epithelial cells in asthma. Int Arch Allergy Immunol 2005;138:142-50.
22. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc 2004;1:176-83.
23. Wilson SJ, Rigden HM, Ward JA, Laviolette M, Jarjour NN, Djukanovic R. The relationship between eosinophilia and airway remodelling in mild asthma. Clin Exp Allergy 2013;43:1342-50.
24. Heijink IH, Kies PM, Kauffman HF, Postma DS, van Oosterhout AJ, Vellenga E. Down-regulation of E-cadherin in human bronchial epithelial cells leads to epidermal growth factor receptor-dependent Th2 cell-promoting activity. J Immunol 2007;178:7678-85.
25. Sweerus K, Lachowicz-Scroggins M, Gordon E, LaFemina M, Huang X, Parikh M, et al. Claudin-18 deficiency is associated with airway epithelial barrier dysfunction and asthma. J Allergy Clin Immunol 2017;139:72-81.e1.
26. Ge Q, Zeng Q, Tjin G, Lau E, Black JL, Oliver BG, et al. Differential deposition of fibronectin by asthmatic bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2015;309:L1093-102.
27. Izuhara K, Ohta S, Ono J. Using periostin as a biomarker in the treatment of asthma. Allergy Asthma Immunol Res 2016;8:491-8.
28. Bains SN, Tourkina E, Atkinson C, Joseph K, Tholanikunnel B, Chu HW, et al. Loss of caveolin-1 from bronchial epithelial cells and monocytes in human subjects with asthma. Allergy 2012;67:1601-4.
29. Tsurikisawa N, Oshikata C, Tsuburai T, Saito H, Sekiya K, Tanimoto H, et al. Bronchial reactivity to histamine is correlated with airway remodeling in adults with moderate to severe asthma. J Asthma 2010;47:841-8.
30. Wight TN. Provisional matrix: a role for versican and hyaluronan. Matrix Biol 2016 [Epub ahead of print].
31. Johnson JR, Roos A, Berg T, Nord M, Fuxe J. Chronic respiratory aeroallergen exposure in mice induces epithelial-mesenchymal transition in the large airways. PLoS One 2011;6:e16175.
32. Bartis D, Mise N, Mahida RY, Eickelberg O, Thickett DR. Epithelial-mesenchymal transition in lung development and disease: does it exist and is it important? Thorax 2014;69:760-5.
33. Lachowicz-Scroggins ME, Yuan S, Kerr SC, Dunican EM, Yu M, Carrington SD, et al. Abnormalities in MUC5AC and MUC5B protein in airway mucus in asthma. Am J Respir Crit Care Med 2016;194:1296-9.
34. Evans CM, Raclawska DS, Ttofali F, Liptzin DR, Fletcher AA, Harper DN, et al. The polymeric mucin Muc5ac is required for allergic airway hyperreactivity. Nat Commun 2015;6:6281.
35. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E, et al. Diminished lipoxin biosynthesis in severe asthma. Am J Respir Crit Care Med 2005;172:824-30.
36. Zehethofer N, Bermbach S, Hagner S, Garn H, Muller J, Goldmann T, et al. Lipid analysis of airway epithelial cells for studying respiratory diseases. Chromatographia 2015;78:403-13.
37. Zhang N, Van Crombruggen K, Gevaert E, Bachert C. Barrier function of the nasal mucosa in health and type-2 biased airway diseases. Allergy 2016;71:295-307.
38. Runswick S, Mitchell T, Davies P, Robinson C, Garrod DR. Pollen proteolytic enzymes degrade tight junctions. Respirology 2007;12:834-42.
39. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest 1999;104:123-33.
40. Persson CG, Erjefalt I, Alkner U, Baumgarten C, Greiff L, Gustafsson B, et al. Plasma exudation as a first line respiratory mucosal defence. Clin Exp Allergy 1991;21:17-24.
41. Tomazic PV, Birner-Gruenberger R, Leitner A, Obrist B, Spoerk S, Lang-Loidolt D. Nasal mucus proteomic changes reflect altered immune responses and epithelial permeability in patients with allergic rhinitis. J Allergy Clin Immunol 2014;133:741-50.

42. Lee HJ, Kim B, Im NR, Lee DY, Kim HK, Lee SH, et al. Decreased expression of E-cadherin and ZO-1 in the nasal mucosa of patients with allergic rhinitis: altered regulation of E-cadherin by IL-4, IL-5, and TNF-alpha. Am J Rhinol Allergy 2016;30:173-8.
43. Steelant B, Farre R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, et al. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. J Allergy Clin Immunol 2016;137:1043-53, e1-5.
44. Bernstein JM, Gorfien J, Noble B, Yankaskas JR. Nasal polyposis: immunohistochemistry and bioelectrical findings (a hypothesis for the development of nasal polyps). J Allergy Clin Immunol 1997;99:165-75.
45. Dejima K, Randell SH, Stutts MJ, Senior BA, Boucher RC. Potential role of abnormal ion transport in the pathogenesis of chronic sinusitis. Arch Otolaryngol Head Neck Surg 2006;132:1352-62.
46. Soyka MB, Wawrzyniak P, Eiwegger T, Holzmann D, Treis A, Wanke K, et al. Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN-gamma and IL-4. J Allergy Clin Immunol 2012;130:1087-96.e10.
47. Schleimer RP. Immunopathogenesis of chronic rhinosinusitis and nasal polyposis. Annu Rev Pathol 2017;12:331-57.
48. Jang YJ, Kim HG, Koo TW, Chung PS. Localization of ZO-1 and E-cadherin in the nasal polyp epithelium. Eur Arch Otorhinolaryngol 2002;259:465-9.
49. Shahana S, Jaunmuktane Z, Asplund MS, Roomans GM. Ultrastructural investigation of epithelial damage in asthmatic and non-asthmatic nasal polyps. Respir Med 2006;100:2018-28.
50. Rogers GA, Den Beste K, Parkos CA, Nusrat A, Delgaudio JM, Wise SK. Epithelial tight junction alterations in nasal polyposis. Int Forum Allergy Rhinol 2011;1:50-4.
51. Meng J, Zhou P, Liu Y, Liu F, Yi X, Liu S, et al. The development of nasal polyp disease involves early nasal mucosal inflammation and remodelling. PLoS One 2013;8:e82373.
52. Shikani AH, Sidhaye VK, Basaraba RJ, Shikani HJ, Alqudah MA, Kirk N, et al. Mucosal expression of aquaporin 5 and epithelial barrier proteins in chronic rhinosinusitis with and without nasal polyps. Am J Otolaryngol 2014;35:377-83.
53. Pothoven KL, Norton JE, Hulse KE, Suh LA, Carter RG, Rocci E, et al. Oncostatin M promotes mucosal epithelial barrier dysfunction, and its expression is increased in patients with eosinophilic mucosal disease. J Allergy Clin Immunol 2015;136:737-46.e4.
54. Pothoven KL, Norton JE, Suh LA, Carter RG, Harris KE, Biyasheva A, et al. Neutrophils are a major source of the epithelial barrier disrupting cytokine oncostatin M in patients with mucosal airways disease. J Allergy Clin Immunol 2016 [Epub ahead of print].
55. Berdnikovs S. Meta-analysis of gene expression microarrays reveals novel biomarkers consistent with altered functionality of mucosal barrier in patients with chronic rhinosinusitis Presented at: 2014 AAAAI Annual Meeting. Milwaukee (WI): AAAAI; 2014.
56. Rosekrans SL, Baan B, Muncan V, van den Brink GR. Esophageal development and epithelial homeostasis. Am J Physiol Gastrointest Liver Physiol 2015;309:G216-28.
57. Odze RD. Pathology of eosinophilic esophagitis: what the clinician needs to know. Am J Gastroenterol 2009;104:485-90.
58. Ravelli AM, Villanacci V, Ruzzenenti N, Grigolato P, Tobanelli P, Klersy C, et al. Dilated intercellular spaces: a major morphological feature of esophagitis. J Pediatr Gastroenterol Nutr 2006;42:510-5.
59. Blanchard C, Stucke EM, Burwinkel K, Caldwell JM, Collins MH, Ahrens A, et al. Coordinate interaction between IL-13 and epithelial differentiation cluster genes in eosinophilic esophagitis. J Immunol 2010;184:4033-41.
60. Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. Mucosal Immunol 2014;7:718-29.
61. Abdulnour-Nakhoul SM, Al-Tawil Y, Gyftopoulos AA, Brown KL, Hansen M, Butcher KF, et al. Alterations in junctional proteins, inflammatory mediators and extracellular matrix molecules in eosinophilic esophagitis. Clin Immunol 2013;148:265-78.
62. Kagalwalla AF, Akhtar N, Woodruff SA, Rea BA, Masterson JC, Mukkada V, et al. Eosinophilic esophagitis: epithelial mesenchymal transition contributes to esophageal remodeling and reverses with treatment. J Allergy Clin Immunol 2012;129:1387-96.e7.
63. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211-21.
64. Paulenda T, Draber P. The role of ORMDL proteins, guardians of cellular sphingolipids, in asthma. Allergy 2016;71:918-30.
65. Bochkov YA, Watters K, Ashraf S, Griggs TF, Devries MK, Jackson DJ, et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad Sci U S A 2015;112:5485-90.
66. Barreto-Luis A, Corrales A, Acosta-Herrera M, Gonzalez-Colino C, Cumplido J, Martinez-Tadeo J, et al. A pathway-based association study reveals variants from Wnt signalling genes contributing to asthma susceptibility. Clin Exp Allergy 2017 [Epub ahead of print].
67. Lavoie-Charland E, Berube JC, Boulet LP, Bosse Y. Asthma susceptibility variants are more strongly associated with clinically similar subgroups. J Asthma 2016;53:907-13.
68. Ober C. Asthma genetics in the post-GWAS era. Ann Am Thorac Soc 2016;13(suppl 1):S85-90.
69. Stefanowicz D, Hackett TL, Garmaroudi FS, Gunther OP, Neumann S, Sutanto EN, et al. DNA methylation profiles of airway epithelial cells and PBMCs from healthy, atopic and asthmatic children. PLoS One 2012;7:e44213.
70. Moheimani F, Hsu AC, Reid AT, Williams T, Kicic A, Stick SM, et al. The genetic and epigenetic landscapes of the epithelium in asthma. Respir Res 2016;17:119.
71. Ji H, Zhang X, Oh S, Mayhew CN, Ulm A, Somineni HK, et al. Dynamic transcriptional and epigenomic reprogramming from pediatric nasal epithelial cells to induced pluripotent stem cells. J Allergy Clin Immunol 2015;135:236-44.
72. Kicic A, Sutanto EN, Stevens PT, Knight DA, Stick SM. Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. Am J Respir Crit Care Med 2006;174:1110-8.
73. Hackett TL, Knight DA. The role of epithelial injury and repair in the origins of asthma. Curr Opin Allergy Clin Immunol 2007;7:63-8.
74. Stremnitzer C, Manzano-Szalai K, Starkl P, Willensdorfer A, Schrom S, Singer J, et al. Epicutaneously applied Der p 2 induces a strong TH 2-biased antibody response in C57BL/6 mice, independent of functional TLR4. Allergy 2014;69:741-51.
75. Vinhas R, Cortes L, Cardoso I, Mendes VM, Manadas B, Todo-Bom A, et al. Pollen proteases compromise the airway epithelial barrier through degradation of transmembrane adhesion proteins and lung bioactive peptides. Allergy 2011;66:1088-98.
76. Balenga NA, Klichinsky M, Xie Z, Chan EC, Zhao M, Jude J, et al. A fungal protease allergen provokes airway hyper-responsiveness in asthma. Nat Commun 2015;6:6763.
77. Kale SL, Agrawal K, Gaur SN, Arora N. Cockroach protease allergen induces allergic airway inflammation via epithelial cell activation. Sci Rep 2017;7:42341.
78. Stentzel S, Teufelberger A, Nordengrun M, Kolata J, Schmidt F, van Crombruggen K, et al. Staphylococcal serine protease-like proteins are pacemakers of allergic airway reactions to Staphylococcus aureus. J Allergy Clin Immunol 2017;139:492-500.e8.
79. Sajjan U, Wang Q, Zhao Y, Gruenert DC, Hershenson MB. Rhinovirus disrupts the barrier function of polarized airway epithelial cells. Am J Respir Crit Care Med 2008;178:1271-81.
80. Rezaee F, DeSando SA, Ivanov AI, Chapman TJ, Knowlden SA, Beck LA, et al. Sustained protein kinase D activation mediates respiratory syncytial virus-induced airway barrier disruption. J Virol 2013;87:11088-95.
81. Hirano M, Tanaka S, Asami O. Classification of polycyclic aromatic hydrocarbons based on mutagenicity in lung tissue through DNA microarray. Environ Toxicol 2013;28:652-9.
82. Friedmann PS, Pickard C. Contact hypersensitivity: quantitative aspects, susceptibility and risk factors. EXS 2014;104:51-71.
83. Tang KT, Ku KC, Chen DY, Lin CH, Tsuang BJ, Chen YH. Adult atopic dermatitis and exposure to air pollutants—a nationwide population-based study. Ann Allergy Asthma Immunol 2017;118:351-5.
84. Huang CC, Wen HJ, Chen PC, Chiang TL, Lin SJ, Guo YL. Prenatal air pollutant exposure and occurrence of atopic dermatitis. Br J Dermatol 2015;173:981-8.
85. van den Bogaard EH, Podolsky MA, Smits JP, Cui X, John C, Gowda K, et al. Genetic and pharmacological analysis identifies a physiological role for the AHR in epidermal differentiation. J Invest Dermatol 2015;135:1320-8.
86. Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, et al. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol 2013;131:736-42.
87. Paulose T, Speroni L, Sonnenschein C, Soto AM. Estrogens in the wrong place at the wrong time: fetal BPA exposure and mammary cancer. Reprod Toxicol 2015;54:58-65.
88. Berdnikovs S, Loffredo L, Abdala-Valencia H. Disruption of the endocrine system leads to local mucosal responses favoring allergic sensitization (MUC2P.919). J Immunol 2015;194(Suppl 1):65.2.

89. Fischer KD, Hall SC, Agrawal DK. Vitamin D supplementation reduces induction of epithelial-mesenchymal transition in allergen sensitized and challenged mice. PLoS One 2016;11:e0149180.
90. Ahrens K, Schunck M, Podda GF, Meingassner J, Stuetz A, Schroder JM, et al. Mechanical and metabolic injury to the skin barrier leads to increased expression of murine beta-defensin-1, -3, and -14. J Invest Dermatol 2011;131:443-52.
91. Hansmann B, Ahrens K, Wu Z, Proksch E, Meyer-Hoffert U, Schroder JM. Murine filaggrin-2 is involved in epithelial barrier function and down-regulated in metabolically induced skin barrier dysfunction. Exp Dermatol 2012;21:271-6.
92. Song H, Yoo Y, Hwang J, Na YC, Kim HS. *Faecalibacterium prausnitzii* subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. J Allergy Clin Immunol 2016;137:852-60.
93. Thuesen BH, Husemoen LL, Hersoug LG, Pisinger C, Linneberg A. Insulin resistance as a predictor of incident asthma-like symptoms in adults. Clin Exp Allergy 2009;39:700-7.
94. Morishita R, Franco Mdo C, Suano-Souza FI, Sole D, Puccini RF, Strufaldi MW. Body mass index, adipokines and insulin resistance in asthmatic children and adolescents. J Asthma 2016;53:478-84.
95. Garmendia JV, Moreno D, Garcia AH, De Sanctis JB. Metabolic syndrome and asthma. Recent Pat Endocr Metab Immune Drug Discov 2014;8:60-6.
96. Berdnikovs S, Abdala-Valencia H, Loffredo LF, Erickson K, Browning M, Saber R, et al. Systemic imbalance in hormone levels associates with epithelial barrier dysfunction in allergic disease [abstract]. J Allergy Clin Immunol 2017;139:AB263.
97. Roarty K, Rosen JM. Wnt and mammary stem cells: hormones cannot fly wingless. Curr Opin Pharmacol 2010;10:643-9.
98. Mullin GE, Eastern JS. Cutaneous signs of thyroid disease. Am Fam Physician 1986;34:93-8.
99. Salazar JJ, Ennis WJ, Koh TJ. Diabetes medications: impact on inflammation and wound healing. J Diabetes Complications 2016;30:746-52.
100. Gunschmann C, Stachelscheid H, Akyuz MD, Schmitz A, Missero C, Bruning JC, et al. Insulin/IGF-1 controls epidermal morphogenesis via regulation of FoxO-mediated p63 inhibition. Dev Cell 2013;26:176-87.
101. Draijer C, Hylkema MN, Boorsma CE, Klok PA, Robbe P, Timens W, et al. Sexual maturation protects against development of lung inflammation through estrogen. Am J Physiol Lung Cell Mol Physiol 2016;310:L166-74.
102. Chen H, Sun X, Chi R, Li X, Feng J, Wu J, et al. Glucocorticoid dexamethasone regulates the differentiation of mouse conducting airway epithelial progenitor cells. Steroids 2014;80:44-50.
103. Sevilla LM, Latorre V, Sanchis A, Perez P. Epidermal inactivation of the glucocorticoid receptor triggers skin barrier defects and cutaneous inflammation. J Invest Dermatol 2013;133:361-70.
104. Shi W, Xu J, Warburton D. Development, repair and fibrosis: what is common and why it matters. Respirology 2009;14:656-65.
105. Elliott EN, Kaestner KH. Epigenetic regulation of the intestinal epithelium. Cell Mol Life Sci 2015;72:4139-56.
106. Adli M, Parlak M, Li Y, El-Dahr SS. Epigenetic states of nephron progenitors and epithelial differentiation. J Cell Biochem 2015;116:893-902.
107. Cavazza A, Miccio A, Romano O, Petiti L, Malagoli Tagliazucchi G, Peano C, et al. Dynamic transcriptional and epigenetic regulation of human epidermal keratinocyte differentiation. Stem Cell Rep 2016;6:618-32.
108. Chanda D, Kurundkar A, Rangarajan S, Locy M, Bernard K, Sharma NS, et al. Developmental reprogramming in mesenchymal stromal cells of human subjects with idiopathic pulmonary fibrosis. Sci Rep 2016;6:37445.
109. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? PLoS Med 2008;5:e62.
110. Batra H, Antony VB. The pleural mesothelium in development and disease. Front Physiol 2014;5:284.
111. Zhou J, Xu F, Yu JJ, Zhang W. YAP is up-regulated in the bronchial airway smooth muscle of the chronic asthma mouse model. Int J Clin Exp Pathol 2015;8:11132-9.
112. Lafkas D, Shelton A, Chiu C, de Leon Boenig G, Chen Y, Stawicki SS, et al. Therapeutic antibodies reveal Notch control of transdifferentiation in the adult lung. Nature 2015;528:127-31.
113. Demehri S, Liu Z, Lee J, Lin MH, Crosby SD, Roberts CJ, et al. Notch-deficient skin induces a lethal systemic B-lymphoproliferative disorder by secreting TSLP, a sentinel for epidermal integrity. PLoS Biol 2008;6:e123.
114. Furmanski AL, Saldana JI, Ono M, Sahni H, Paschalidis N, D'Acquisto F, et al. Tissue-derived hedgehog proteins modulate Th differentiation and disease. J Immunol 2013;190:2641-9.
115. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, et al. Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. J Allergy Clin Immunol 2014;133:1390-9, e1-6.
116. Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie AN, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. J Allergy Clin Immunol 2016;138:1356-66.
117. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008;178:667-72.
118. Larkin EK, Gebretsadik T, Moore ML, Anderson LJ, Dupont WD, Chappell JD, et al. Objectives, design and enrollment results from the Infant Susceptibility to Pulmonary Infections and Asthma Following RSV Exposure Study (INSPIRE). BMC Pulm Med 2015;15:45.
119. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate immunity and asthma risk in Amish and Hutterite farm children. N Engl J Med 2016;375:411-21.
120. Davies DE, Holgate ST. Asthma: the importance of epithelial mesenchymal communication in pathogenesis. Inflammation and the airway epithelium in asthma. Int J Biochem Cell Biol 2002;34:1520-6.
121. Hackett TL. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. Curr Opin Allergy Clin Immunol 2012;12:53-9.
122. Hackett TL, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky DV, Murray LA, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factor-beta1. Am J Respir Crit Care Med 2009;180:122-33.
123. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. J Allergy Clin Immunol 2011;128:549-56, e1-12.
124. Cheng E, Souza RF, Spechler SJ. Tissue remodeling in eosinophilic esophagitis. Am J Physiol Gastrointest Liver Physiol 2012;303:G1175-87.
125. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. J Allergy Clin Immunol 2014;134:509-20.
126. Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march. J Allergy Clin Immunol 2016;138:350-8.e1.
127. Kelleher MM, Dunn-Galvin A, Gray C, Murray DM, Kiely M, Kenny L, et al. Skin barrier impairment at birth predicts food allergy at 2 years of age. J Allergy Clin Immunol 2016;137:1111-6, e1-8.
128. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. Pediatrics 2001;108:E33.
129. Alduraywish SA, Standl M, Lodge CJ, Abramson MJ, Allen KJ, Erbas B, et al. Is there a march from early food sensitization to later childhood allergic airway disease? Results from two prospective birth cohort studies. Pediatr Allergy Immunol 2017;28:30-7.
130. Alduraywish SA, Lodge CJ, Campbell B, Allen KJ, Erbas B, Lowe AJ, et al. The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies. Allergy 2016;71:77-89.
131. Brunner PM, Silverberg JI, Guttman-Yassky E, Paller AS, Kabashima K, Amagai M, et al. Increasing comorbidities suggest that atopic dermatitis is a systemic disorder. J Invest Dermatol 2017;137:18-25.
132. Liu W, Huang C, Wang X, Cai J, Hu Y, Zou Z, et al. Multimorbidities of asthma, allergies, and airway illnesses in childhood: chance or not chance? J Asthma 2016;1-12.
133. Williamson PA, Vaidyanathan S, Clearie K, Barnes M, Lipworth BJ. Airway dysfunction in nasal polyposis: a spectrum of asthmatic disease? Clin Exp Allergy 2011;41:1379-85.
134. Wang Z, Zhang LJ, Guha G, Li S, Kyrylkova K, Kioussi C, et al. Selective ablation of Ctip2/Bcl11b in epidermal keratinocytes triggers atopic dermatitis-like skin inflammatory responses in adult mice. PLoS One 2012;7:e51262.
135. Fallon PG, Sasaki T, Sandilands A, Campbell LE, Saunders SP, Mangan NE, et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet 2009;41:602-8.
136. Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol 2001;117:977-83.
137. Weber S, Niessen MT, Prox J, Lullmann-Rauch R, Schmitz A, Schwanbeck R, et al. The disintegrin/metalloproteinase Adam10 is essential for epidermal integrity and Notch-mediated signaling. Development 2011;138:495-505.
138. Allen JE, Wynn TA. Evolution of Th2 immunity: a rapid repair response to tissue destructive pathogens. PLoS Pathog 2011;7:e1002003.
139. Gause WC, Wynn TA, Allen JE. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nat Rev Immunol 2013;13:607-14.

140. Huang L, Beiting DP, Gebreselassie NG, Gagliardo LF, Ruyechan MC, Lee NA, et al. Eosinophils and IL-4 support nematode growth coincident with an innate response to tissue injury. PLoS Pathog 2015;11:e1005347.
141. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in health and disease: the LIAR hypothesis. Clin Exp Allergy 2010;40:563-75.
142. Kypriotou M, Huber M, Hohl D. The human epidermal differentiation complex: cornified envelope precursors, S100 proteins and the 'fused genes' family. Exp Dermatol 2012;21:643-9.
143. van Drongelen V, Alloul-Ramdhani M, Danso MO, Mieremet A, Mulder A, van Smeden J, et al. Knock-down of filaggrin does not affect lipid organization and composition in stratum corneum of reconstructed human skin equivalents. Exp Dermatol 2013;22:807-12.
144. Folmes CD, Terzic A. Energy metabolism in the acquisition and maintenance of stemness. Semin Cell Dev Biol 2016;52:68-75.
145. Naquet P, Giessner C, Galland F. Metabolic adaptation of tissues to stress releases metabolites influencing innate immunity. Curr Opin Immunol 2016;38: 30-8.
146. Ryall JG, Cliff T, Dalton S, Sartorelli V. Metabolic reprogramming of stem cell epigenetics. Cell Stem Cell 2015;17:651-62.
147. Galvis LA, Holik AZ, Short KM, Pasquet J, Lun AT, Blewitt ME, et al. Repression of Igf1 expression by Ezh2 prevents basal cell differentiation in the developing lung. Development 2015;142:1458-69.
148. Klok E, Lubsen NH, Chamberlain CG, McAvoy JW. Induction and maintenance of differentiation of rat lens epithelium by FGF-2, insulin and IGF-1. Exp Eye Res 1998;67:425-31.
149. Maki RG. Small is beautiful: insulin-like growth factors and their role in growth, development, and cancer. J Clin Oncol 2010;28:4985-95.
150. Ijaz T, Pazdrak K, Kalita M, Konig R, Choudhary S, Tian B, et al. Systems biology approaches to understanding epithelial mesenchymal transition (EMT) in mucosal remodeling and signaling in asthma. World Allergy Organ J 2014;7: 13.
151. Kalita M, Tian B, Gao B, Choudhary S, Wood TG, Carmical JR, et al. Systems approaches to modeling chronic mucosal inflammation. Biomed Res Int 2013; 2013:505864.
152. Wawrzyniak P, Wawrzyniak M, Wanke K, Sokolowska M, Bendelja K, Ruckert B, et al. Regulation of bronchial epithelial barrier integrity by type 2 cytokines and histone deacetylases in asthmatic patients. J Allergy Clin Immunol 2017;139: 93-103.