Epithelial Barrier: Protector and Trigger of Allergic Disorders

Short running title: Epithelial Barrier and Allergy

Izquierdo E1, Rodriguez-Coira J1, Delgado-Dolset MI1, Gomez-Casado C1,2, Barber D1, Escribese MM1

1IMMA, Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo CEU, Madrid, Spain.
2Department of Dermatology, University Hospital Düsseldorf, Düsseldorf, Germany.

Corresponding author:
Maria M. Escribese
Universidad CEU San Pablo
Facultad de Medicina
Urb. Montepríncipe s/n,
28668 Madrid, Spain.
mariamarta.escribesealonso@ceu.es

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0779
ABSTRACT

The epithelial barrier has been classically considered as only the first line of defense against irritants, pathogens, and allergens, but it is now known that it also plays an essential role in the immunological response against exogenous agents. In fact, recent reports postulate the epithelial barrier hypothesis as a possible explanation for the increasing incidence and severity of allergic diseases.

The epithelial barrier preserves the isolation of the inner tissues from potential external threats. Moreover, a coordinated interaction between epithelial and immune cells ensures the unique immune response taking place in mucosal tissues and that is has been reported to be dysregulated in allergic diseases.

Herein, we and others have demonstrated that in severe allergic phenotypes, the epithelial barrier experiments several histological modifications and increased in immune cells infiltration, leading to its dysfunction. This is common in atopic dermatitis, asthma, and/or food allergy. However, the precise role of the epithelial barrier in the mucosal biology during allergic diseases progression is not well understood yet.

In this review, we aim to compile recent knowledge regarding the histological structure and immunological function of the epithelial barrier and to shed light on the role of this compartment in the onset, and progression of allergic diseases.

Key words: Epithelium. Mucosal immunity. Allergy. Antigen. Immunoglobulin (Ig) switch. Inflammation.
RESUMEN

La barrera epitelial se ha considerado clásicamente sólo como la primera línea de defensa contra los irritantes, patógenos y alérgenos, pero ahora sabemos que el epitelio también desempeña un papel esencial en la respuesta inmunológica frente los agentes exógenos. De hecho, informes recientes postulan la hipótesis de la barrera epitelial como una posible explicación de la creciente incidencia y la gravedad de las enfermedades alérgicas. La barrera epitelial preserva el aislamiento de los tejidos internos de las posibles amenazas exteriores. Se sabe que las células epiteliales además de un papel meramente protector también tienen una función esencial en el desarrollo de la respuesta inmune en las mucosas, favoreciendo un ambiente tolerogénico. Sin embargo, en enfermedades alérgicas estas características se ven afectadas como demuestra una respuesta exagerada ante antígenos inocuos. De hecho, en los fenotipos alérgicos graves, la barrera epitelial experimenta varias modificaciones histológicas que se asocian con pérdida de integridad y aumento de los infiltrados celulares, lo que conduce a una disfunción de la misma. Este proceso es común en la dermatitis atópica, el asma y/o la alergia alimentaria. Aunque, todavía no se conoce bien la función exacta de la barrera epitelial en la biología de la mucosa durante las enfermedades alérgicas.

En esta revisión, pretendemos recopilar los conocimientos recientes sobre la estructura histológica y la función inmunológica de la barrera epitelial y arrojar luz sobre el papel de este compartimento en la aparición y la progresión de las enfermedades alérgicas.

1. INTRODUCTION

The mucosa lines body cavities and passages (as of the gastrointestinal, respiratory, reproductive or urinary tract), which communicate directly or indirectly with the outside of the body. In general, mucosal tissues have two main features: 1) a specific histological conformation that preserves isolation of the inner tissues from the potential outside harms, and 2) a privileged immunity, which together allow a particular and tight regulation of the immune response that is essential for homeostasis maintenance and participates in the development of several diseases, including allergic diseases and asthma.[1] Mucosal tissues are usually composed of conjunctive tissue and epithelium.

Epithelial tissue in the mucosa is in contact with the outside and underlined by a basal membrane and a lamina propria formed by connective tissue, which is highly vascularized and composed of different cell types (stromal and immune cells) and extracellular matrix proteins. There are several types of mucosal tissues depending on their location in the body: oral, respiratory, skin or gastrointestinal. Each one displays specific histological features[2–4].

The epithelium has been classically considered as only the first line of defense against inhaled irritants, pathogens and allergens, but since a few years ago epithelial cells have been shown to play an essential role in the immunological response and during the inflammatory process after tissue damage[5,6]. The epithelium consists of cells tightly attached to each other and arranged in several distinct layers. Junctional complexes like tight junctions (TJs), adherent junctions (AJs), gap junctions and desmosomes provide cohesion between cells. TJs form the closest cell-cell interactions in the apical area of oral epithelial cells, working as a restrictive gate for the passage of water, electrolytes, and other small molecules. They consist of different transmembrane proteins, including occludin, claudin and immunoglobulin-like surface proteins, as well as cytoplasmic molecules such as zonula occludens (ZO)[7]. AJs are protein complexes situated bellow TJs that strongly hold cells. AJs are composed by cadherins that connect to the actin cytoskeleton[8]. Gap junctions are composed of hemichannels, called connexons, which are regulated by several factors including pH, calcium concentration and posttranslational modifications. Thus, they provide direct communication between adjacent cells and regulate the exchange of small molecules and ions. Finally, desmosomes link two cells
together by the intermediate filament cytoskeleton, becoming the adhesive bonds that
give mechanical strength to tissues. Since the structure and functions of all
abovementioned cell-cell junctions are key to preserve epithelial barrier integrity, their
disruption has been linked to infections, autoimmune diseases, allergy, and cancer
disorders[9].

1.1. Respiratory system

The respiratory system is divided anatomically into structures of the upper and the lower
respiratory tract, that correspond to two functional components: the conductive and the
respiratory, respectively.

The conductive portion, that involves the nasal cavity, pharynx, larynx and trachea, is
lined by a mucosal tissue formed by ciliated pseudostratified columnar epithelium. While
deep into the respiratory system, the epithelium becomes thinner, and the conformation
of glands is less specialized and more abundant, passing from olfactory glands in the nasal
cavity to seromucous glands in the trachea. These histological features in the upper
airways allow the isolation of the inner tissues and the moisturizing and conduction of the
air into the lower respiratory tract, the respiratory portion.

The trachea bifurcates into the right and left primary bronchi that enter the posterior side
of each lung along with the pulmonary vessels, lymphatics, and nerves. Within each lung,
each bronchus subdivides further to form the bronchial tree, the last component of the air
conductive system. It is in the last parts of this portion, in the alveoli, where the gas
exchange between air and blood occurs at the membrane barrier between each alveolus
and the capillaries surrounding it.

The respiratory epithelium provides physical barrier to infection, lining the respiratory
tract from the nose to the alveoli with a wide range of cell types. Ciliated epithelial cells
are important in propelling mucus up the airway, thereby removing particulate material.
Ciliated cells line the respiratory tract down to the level of the respiratory bronchiole.
Tracheobronchial glands are important sources of airway mucus, which serves to trap
particulates. The respiratory epithelium also functions in the regulation of water and ion
movement into the airway mucus. It secretes surfactant proteins-A (SP-A) and -D (SP-
D), lysozyme, lactoferrin, and antimicrobial peptides (β-defensins and cathelicidins), and releases reactive oxygen and nitrogen species to kill invading pathogens[5,10–13].

1.2. Oral and gastrointestinal tract

The gastrointestinal track covers the oral cavity, esophagus, stomach, intestine an anus in addition to associated glands. Its main function is to obtain the molecules necessary for the maintenance, growth, and energy needs of the body from ingested food.

Histologically, it is formed by four main layers: mucosa, submucosa, muscularis and serosa. We will focus on special attention in the epithelial tissue that forms the mucosa layer[11]. The gastrointestinal tract entrance is the oral cavity. Here, the mucosal structure varies along its location within the oral cavity, but three main types of mucosa can be recognized based on their morphology and specific pattern of differentiation: 1) keratinized stratified squamous epithelium or masticatory mucosa, which covers the hard palate and gingiva; 2) non-keratinized stratified squamous epithelium or lining mucosa, on the underside of the tongue, inside of the lips, cheeks, floor of the mouth, and alveoli; and 3) the specialized mucosa of the dorsal surface of the tongue[9,10,14]. The oral epithelium is the superficial layer that separates environment from underlying tissues. It is a stratified squamous epithelium consisting of cells tightly attached to each other and arranged in layers. It possesses structural properties, like stratification and cornification of the keratinocytes, and specific cell-to-cell interactions to maintain its barrier function. The keratinized type contains four layers of cells: the basal layer, spinous layer, granular layer, and the superficial layer (keratinized layer). Keratinocytes are born and proliferate in the basal layer and undergo terminal differentiation as they migrate to the surface, where they die. Thus, the outermost cell layers are composed by dead cells. Conversely, the surface cells of non-keratinized epithelia are living cells without keratin. Besides, non-keratinized oral epithelium has no granular layer[10,15].

The oral cavity is connected with the pharynx (oropharynx), followed by the esophagus, both characterized by stratified squamous epithelium which ends in the stomach, where the stratified squamous epithelium lining is replaced by simple columnar epithelium with glands and the intestine[11].
The small intestine is the site where the digestive processes are completed, and where the nutrients (production of digestion) are absorbed by cells of the epithelial lining. It has been deeply studied due to its immune-privileged features. In the small intestine, the epithelium from the mucosal layer is made up by simple columnar epithelium with microvilli, aiming to increase the surface of absorption, and goblet cells that are responsible for mucus secretion. However, in the large intestine, the mucosa lacks microvilli, but still presents goblet cells and absorptive cells involved in water and electrolytes absorption. [16–18].

1.3 The skin

The skin is the biggest tissue in the body and is mainly conformed by three layers called epidermis, dermis, and hypodermis[11].

The epidermis presents a stratified squamous epithelium mainly formed by keratinocytes. It is the most external layer of the skin and displays a high regeneration rate in response to exogenous factors (detergents, environmental pollutants, etc.). Other less abundant cell types in the epidermis are melanocytes and Langerhans cells. The formers are involved in the immune response taking place in this tissue. The epidermis is organized in several layers called stratum. The most external layer is the stratum corneum, made up of dead keratinocytes. Next is the stratum lucidum, a very thin layer mainly presents in areas like the hand palms or feet sole. The third layer is the stratum granulosum, formed by rhomboid cells containing big granules of keratin, which is followed by the stratum spinosum, a layer formed by polygonal cells with remarkable intracellular adhesions. Finally, the inner layer is the stratum basal, which contains a few cell layers of cubic cells with a high proliferative potential[11].

Beneath the epidermis is located the middle layer of the skin, called dermis. This layer mainly comprises connective tissue fibers (e.g., collagen, elastin).

The last layer of the skin is the hypodermis, that is predominantly constituted by loose connective tissue, what makes possible for the skin to slide over subjacent organs.
2. MUCOSAL IMMUNE SYSTEM

The need for permeability in the epithelial lining of mucous membranes (food absorption, gas exchange, reproduction) creates vulnerability to pathogens, making these regions the gateway to many infectious agents. Nonetheless, mucosa tissues rely on the larger part of the immune system that comprises mucus layers, epithelium cells, lymphoid tissues and immune molecules that are situated in the mucosal membranes of the gut, the respiratory system and the urogenital tract, providing a first defense line of the inner body surfaces.

Mucosal tissues are continuously exposed to external stimuli, what could lead to endless systemic proinflammatory responses, but the mucosal immune system counts on two strategies to preserve homeostasis: 1) immune exclusion by secretory IgA antibodies, and 2) a confined tolerogenic immune response. Immunoglobulins (Igs) are the first barrier defense of mucosa tissues, from which polimeric IgA (pIgA) is the predominant isotype, except in the lower respiratory and genital tracts, where IgG is the major one[19,20]. Secreted IgA is synthesized by plasma cells derived from activated B cells in the lamina propia (Figure 1). Then, the antibody binds the pIg receptor (pIgR) expressed on the basolateral surfaces of epithelial cells, what helps pIgA internalization, transport to the apical side and release to the lumen. Subsequently, pIgA blocks the access of antigens and pathogens to the epithelial barrier and enhances their agglutination[19,21]. Microorganisms get trapped in the mucus layer to be later eliminated by peristaltic and mucociliary events[22].

Alterations in the epithelial barrier integrity could lead to pathogen invasion. To avoid it, the mucosal immune system comprises inductive and effector sites where the immune response is initiated and manifested, respectively. The response to microorganisms is initiated by epithelial cells and antigen presenting cells (APCs). The epithelial barrier is situated underneath the mucus layer, and contains different types of specialized epithelial cells that vary according to the organ and contribute to mucosal immune regulation[16] (Figure 2). Goblet cells secrete mucins and antimicrobial peptides, which play a critical role in antigen transfer from luminal to lamina propria, contributing to food tolerance [23]. Additionally, the human airway epithelium contains another secretory type of cell, the club cells, which produce glycoproteins, lipids, and peptides to provide chemical and physical airway protection[24,25]. Different studies have shown alterations of club cells
in inflammatory pathologies, such as allergy[26]. Besides, in response to epithelial injury, club cells differentiate into ciliated and goblet cells[24]. Paneth cells are located in the epithelium of the small intestinal, and they sustain homeostasis by releasing antimicrobial peptides and proteins that regulate the amount of commensal and pathogenic microorganisms[27,28] (Figure 2). Additionally, different endocrine cells (e.g., enteroendocrine and neuroendocrine cells) are found along the epithelial barrier, and they may secrete a wide range of peptide hormones and neuropeptides, such as serotonin, vasoactive intestinal peptide and GABA (gamma-aminobutyric acid), that influence immune cells functions[29,30] (Figure 2). Distributed along mucosal epithelium, there are also individual chemosensory cells called tuft cells, also known as brush cells or microvillus cells (Figure 2). They contribute to expulse helminths and generate a type 2 immune response[31]. Tuft cells produce IL-25 and stimulate the development of innate lymphoid cells type 2 (ILC2s), that could lead to intestinal eosinophilia[32,33]. However, the specific role of tuft cells in allergy remains to be clarified. Another highly specialized cell is the Microfold (M) cell, which is a unique epithelial subtype that overlies the lymphoid tissue and plays a role in transepithelial antigen transport (Figure 2). M cells take up luminal microbes through phagocytosis, endocytosis, or transcytosis and deliver them to dendritic cells located in the lamina propria (Figure 2). Moreover, M cells can express IgA receptor on their apical surface to bind and transport secretory IgA-bound antigens[19,34]. This antigen-transport function suggest that M cells may contribute to the genesis of allergy. Finally, epithelial cells present supplementary immune-regulatory features, like class I and II major histocompatibility complex (MHC) expression[35], which allows them to present antigens to T cells, concomitant to CD23 expression (the low-affinity receptor for IgE, FcεRII), that allows them to transport IgE and IgE-immune complexes across the epithelial monolayer[36]. Although robust evidence supports a role of epithelial cells in mucosal immune response, studies focusing on the specific involvement of individual epithelial cell subtypes in allergy are scarce.

Apart of the abovementioned luminal-antigen transference strategies performed by epithelial cells, antigens can be directly taken up by dendritic cells (DCs) with extending trans-epithelial dendrites and their own TJ proteins[37] (Figure 3). When the epithelial barrier is not damaged, antigen entry occurs at specific sites underneath lymphoid follicles mainly constituted of clustered B-cell follicles interspersed with T-cell zones and a
variety of APCs cells. This organized structure is called the mucosa-associated lymphoid tissue (MALT), and together with local lymph nodes, it is where mucosal immune system is initiated (Figure 3)[38]. MALT is classified according to their body location as gut-associated lymphoid tissue (GALT), bronchial/tracheal-associated lymphoid tissue (BALT), nose-associated lymphoid tissue (NALT), and vulvovaginal-associated lymphoid tissue (VALT).

Epithelial cells and APCs are informed of the presence of microbes via different pattern recognition receptors (PPRs) (Figures 1 and 3). Not all the microorganisms are pathogenic, as it is the case of microbiota. Thus, polar expression (apical versus basolateral side) of PPRs is crucial for preventing unnecessary inflammatory responses. Under homeostatic conditions, microbiota interaction induce transforming growth factor-β (TGF-β), retinoic acid and thymic stromal lymphopoietin (TSLP), which promote tolerogenic APCs and, together with IL-10, the induction of regulatory T cells (Tregs) [39,40]. Tolerogenic APCs and Tregs stimulate B cell class switching to IgA production, which after maturation generate plasma cells able to migrate to the mucosa[41,42]. To achieve a successful IgA switch, type 3 innate lymphoid cells (ILC3) need to provide lymphotoxin-β receptor-dependent signals to DCs and stromal cells[43]. ILC3s play a major role in maintaining tolerance in the gut mucosa by stimulating B-cell differentiation, the development of isolated lymphoid follicles and the production of IL-22, IL-17 and GM-CSF (granulocyte macrophage colony-stimulating factor). [44]. A newly found regulatory ILC (ILCreg) has been shown to participate in preserving gut homeostasis via the secretion of IL-10 and TGF-β and relieving intestine inflammation[45]. The role of type 1 and 2 ILCs (ILC1s and ILC2s, respectively) in the healthy gut is to date unclear. On the contrary, both subtypes are key cells to preserve epithelial barrier under pathogenic conditions[46,47].

Upon sampling antigens, conventional DCs (cDCs) migrate to draining lymph nodes to present them to naïve T lymphocytes (Figure 3). Depending on the antigen source and the cytokine environment, activated cDCs can polarize naïve T cells to T helper cells (Th) Th1, Th17, Th2 or Th9 phenotype[48] (Figure 3). Pathogens that accumulate inside cDCs vesicles stimulate the differentiation of Th1 cells, whereas the prototypical response to helminths is characterized as Th2[49]. Segmented filamentous bacteria produce Th17
cells differentiation in the small intestine[50], and the commensal *Staphylococcus aureus* and the opportunistic fungus *Candida albicans* have been suggested to induce polarization of Th9 cells[51]. Once T cell are polarized, and together with antigen-activated cDCs, they promote class switching of B cells, which prevent the spreading of the infection. Then, B and T cells migrate to the mucosa effector sites, where B and T cells express their effector functions, mainly releasing immunomodulatory cytokines.

In the event of infection, different types of immune cells are recruited through specialized vascular structures that allow lymphocytes to migrate into the tissue[52]. Bacterial and viral infections trigger type 1 response (Th1 cells, natural killer cells, ILC1, Th17 cells and ILC3 activation) that includes the release of proinflammatory cytokines (IL-1β, IL-18, IFN-γ)[53] and cytokines with protective functions (IL-23, IL-22 and IL-17)[54,55]. Then, neutrophiles are recruited to produce a wide range of proinflammatory and anti-inflammatory effector molecules[56], that contribute to the recruitment of other immune cells to control pathogen spreading and later release factors necessary for the resolution of inflammation.

On the contrary, tick, insect, snake bites and stings, and helminth invasions trigger a type 2 response (ILC2, Th2 cells, eosinophils, basophils and mast cells activation) that is initiated by tuft cells by releasing alarmins (TSLP, IL-25, and IL-33) that stimulate the production of IL-4, IL-5, and IL-13 and enhance B-cell class switching to IgE[57,58]. Similarly, allergic patients display a type 2 inflammatory response that is initiated after allergen exposure and results in the production of allergen specific IgE, which might lead to airway hyperresponsiveness and the production of mucus[6,59].

Additionally, the mucosa immune system counts on intraepithelial γδ-T cells, T cells the express the T cell receptor-γδ (TCR-γδ) and are especially located in the epithelia of mucosal tissues. These T-cells scan for signs of cellular stress and respond with rapid effector functions (*e.g.*, lysing cells) when they detect infected or transformed host cells, or if they sense critical information from other mucosal leukocyte populations. Recent data suggest that γδ-T cells might be implicated in allergic airway diseases (asthma, rhinitis) and in intestinal hypersensitivity processes (food allergy, celiac disease) but its significance is not well known yet[60,61].
3. EPITHELIAL BARRIER REMODELING IN ALLERGY

Allergic rhinitis, which was already common in the late 19th century, allergic asthma, and atopic dermatitis, which reached epidemic proportions after the 1960s[62–64], and food allergy, eosinophilic esophagitis, and drug-induced anaphylaxis, considered epidemic since 2000, are common allergic diseases in industrialized areas[65–67]. Many allergens derived from environmental agents such as dust mites, bacteria, fungi, viruses, and toxins are daily encountered by human as a consequence of industrialization. In addition, human skin and mucosa are daily exposed to substances commonly used for laundry or household cleaning, such as detergents or surfactants, enzymes and emulsifiers in processed food, cigarette smoke, particulate matter, diesel exhaust, ozone, nanoparticles and microplastics. These agents have been proven toxic for epithelial cells[68–71] (Figure 4).

The ability of the epithelium to control the balance between tissue damage and repair signals is essential to limit epithelial damage and subsequent inflammation and disease development[72]. When the remodeling of epithelial barriers leads to increased leakiness, it also causes microbial dysbiosis and the translocation of bacteria to subepithelial areas, which induces tissue microinflammation[73] and sustained T cell activation, being these alterations a potential mechanism behind the onset of diverse allergic diseases[68].

Atopic dermatitis (AD) is an inflammatory skin disorder that affects 25% of children and 10% of adults, and which possesses a higher risk of allergic rhinitis and asthma later in life. Filaggrin mutations and TJs deficiency (claudin-1, claudin-4, and claudin-6) have been described in the skin of patients with AD[74–80]. A recent gene expression analysis by RNA sequencing performed on matched lesional and non-lesional skin tissue biopsies from AD patients and healthy subjects revealed that cell adhesion, cadherin signaling, and keratinization are the most differentially expressed gene groups in patients with AD. Of those, gene expression levels of CLDN4 and TJP1 negatively correlated with Staphylococcus aureus in lesional samples[81]. A decrease in the skin microbiota diversity due to an increased S. aureus abundance has been linked with AD severity[81,82]. The role of S aureus is allergy diseases in controversial. A recent study on asmatic patients showed that sensitization to S aureus enterotoxin B was associated with the presence of AD and with an increased risk of sensitization to common
aeroallergens[83]. The complex interaction between the skin microbiota, the epithelial barrier and the immune system is increasingly clear to be key in understanding the development of AD.

AD is primarily a Th2 cell-driven disease, with variable numbers of eosinophils, and where IgE is considered to play just a bystander role. Targeting the Th2 signature cytokines IL-4 and IL-13 with biological antagonists has been proven efficient in AD treatment. This indicates that these cytokines are major players in inducing skin inflammation in AD[84–88]. In contrast, studies targeting the epithelial alarmins TSLP and IL-33 have not shown any effects on AD yet[89,90]. Emerging evidence shows that mast cells, eosinophils, and basophils are pivotal effector cells in causing pruritus in AD[91,92]. Chronic pruritus is a major clinical complaint in AD, since it severely reduces the quality of life of the AD patients and is difficult to treat[93]. Basophils and eosinophils infiltrate the skin in AD[94], and locate in close proximity to the nerves, providing a bridge between the neuronal and immune systems, and amplifying local immune reactions[95]. Recent findings underscore that skin barrier defects may support pruritus induction[96]. In AD, chronic scratching worsens clinical symptoms. The impaired barrier function associated with the itch-scratch cycle further augments this positive feedback loop. IL-31 has recently emerged as one of the most efficient pruritus treatment approaches in AD[97–100].

Allergic asthma is a heterogeneous lung disease characterized by chronic airway inflammation. It produces remodeling of the airway respiratory mucosa causing airway obstruction and the subsequent loss of respiratory function[101,102]. Upon contact with a trigger (e.g., viral infections, allergens or pollution) asthma gets exacerbated[103]. Due to the ubiquitous presence of triggers and the heterogeneity of asthma patients, exacerbations cannot be fully prevented[104]. Airway mucosa remodeling in asthma can affect different layers of the epithelial barrier: the airway smooth muscle (ASM) below the epithelium, the extracellular matrix (ECM) and epithelial basal line, the epithelial layer itself and even the lumen[105,106]. The ASM tends to thicken with chronic inflammation causing hypertrophy and hyperplasia, lowering the contractile potential of the airway tissue and hence diminishing function[107,108]. This process often goes hand
in hand with an increase in deposition of the ECM, which has been linked to an increase of arginase activity that liberates precursors for nitric oxide (NO) synthesis[109]. This process can be repressed by endogenous compounds like dimethylarginine (ADMA), which inhibit the NO synthase (NOS)[109,110]. Interestingly, recent research has shown that in vitro culture of healthy epithelia incubated with Der p 1 produced higher amounts of ADMA in comparison with damaged epithelia[111]. Moreover, Der p 1 is a known proteolytic allergen that can cleave and disrupt TJs[112]. This disruption of TJs in the epithelial layer not only dysregulates the epithelial cell differentiation but also allows the entrance of other allergens and pathogens, which can then trigger continuous exacerbations[113–115]. This process is characterized by the decreased expression of structural proteins forming the TJs such as occludin, zonula occludens and claudin-18 in the epithelial cells[116,117]. Nevertheless, continuous release of histamine, IL-4 and TNF-α by basophils and T cells, respectively, also promote epithelial permeabilization in primary human bronchial epithelial cells (HBECs) and mouse models. This suggests that chronic Th2 allergic response as the main driver of the phenotype[118]. The loss of TJs is associated with changes in the cell type composition of the epithelial layer. During remodeling there is an increase in the number of goblet cells, which induces mucus overproduction and secretion to the airway lumen, reduces the O₂ exchange and obstructs the airway lowering the respiratory function[68]. It has recently been discovered that microRNA 141 (miRNA141) may play a key role in this process. This miRNA is abundantly detected in the human airway epithelium and its expression can be induced upon airway allergen challenge in asthma. Strikingly, the inhibition of miRNA141 lowered airway hyperreactivity and suppressed mucus overproduction by IL-13 signaling[119].

Chronic inflammatory diseases can also influence the structure of the nasal mucosa. This is evident in chronic rhinosinusitis with nasal polyps (NP) in which the normal mucosa suffers a remodeling process comprised of rupture of the epithelial layer, proliferation of fibrotic tissue, ECM deposition with edema, infiltration of immune cells and thin-wall vessels[120–122]. In this process, Th2 related cytokines such as IL-4, IL-5 and IL-13 play a key role by recruiting eosinophils to the area, which would be responsible for causing the edema and maintaining the inflammatory cascade over time[123,124]. Interestingly, recent research suggests that the epithelial basal cells may also play an important role in
this process. Ordovas-Montanes et al.[125] proposed that basal cells derived from NP may play a role in the reappearance of NP. In contrast with basal cells derived from healthy nasal mucosa, which maintain constitutively the expression of Wnt pathway in NP, Wnt genes are mostly induced by IL-4/IL-13 and limit basal cell differentiation to secretory cells. The authors showed that NP basal cells could maintain Wnt expression without IL-4, IL-13 presence mirroring an inflammatory “memory” phenotype, inhibiting their differentiation, and promoting NP relapse[126].

Another disease in which remodeling plays an important role is eosinophilic esophagitis (EoE), an antigen driven Th2 disease in which chronic eosinophilic inflammation causes esophageal dysfunction[126,127]. EoE is mainly driven by food allergens; however, certain aeroallergens can also initiate the disease[128–131]. Interestingly, IgG4, instead of IgE, appears to be the main driver of the disease, as the treatment with omalizumab (anti-IgE) was not effective, and deposits of IgG4 and IgG4-expressing plasma cells were observed in EoE biopsies[132]. EoE is defined by edema, exudates, longitudinal furrows and esophageal narrowing in advanced disease. All these changes ultimately cause epithelial barrier dysfunction. At the structural level, the loss of several structural proteins such as desmoglein 1, E-cadherin, occludin, claudins 1 and 7 lead to decreased numbers of desmosomes and other intercellular junctions[133–136]. This promotes an epithelial to mesenchymal transition (EMT) in which epithelial cells transdifferentiate to fibrotic cells causing the remodeling of the mucosal layer[137].

Finally, little is known about the effect of food allergy in the remodeling of different mucosal layers. Recent reports show that severe allergic respiratory individuals living in high allergen exposure areas present remodeling in the oral mucosa mediated by allergic reactions characterized by impaired TJs formation, immune cell infiltration and ECM over production[138,139]. This suggests that oral mucosa might play a key role in allergic sensitization, tolerance and mucosal remodeling. This notion is supported by the tolerogenic properties of the secondary organs linked to the oral mucosa (tonsils) and the success of oral allergen immunotherapy (AIT)[140–142]. However, gut mucosal remodeling by food allergy is a very unexplored field, probably due to the continuous avoidance of antigen exposure as a common treatment. Nevertheless, it is hypothesized that the interplay between the gut microbiota and the intestinal eosinophils may cause
intestinal alterations in the epithelial barrier[143]. In any case, these processes should be further investigated (Figure 5).

4. EFFECT OF THE INFLAMMATORY RESPONSE ON THE EPITHELIAL BARRIER

Once the inflammation has fulfilled its purpose, it needs to be controlled to prevent chronic inflammation. Thus, there is a change in pro-inflammatory factors, responsible of the activation of immune response, towards a pro-resolving environment, which is achieved by the production of pro-resolving agents (Figure 6).

Pro-resolving factors are anti-inflammatory mediators that meet a series of requirements,[144] including: 1) limit the recruitment, infiltration and activation of leukocytes (particularly neutrophils, which are the first to enter the inflammatory site and start the phagocytosis of microbes[145]); 2) promote the recruitment of non-inflammatory (non-phlogistic) immune cells; 3) induce the apoptosis and clearance of neutrophils (efferocytosis), as well as 4) inhibit pro-inflammatory mediators such as cytokines; 5) induce an anti-inflammatory status; and 6) activate tissue regeneration and healing, restoring homeostasis[146,147]. Resolution of inflammation is tissue-specific, having general characteristics and organ-specific ones[145] that could explain differences in regeneration among locations (e. g. skin, which suffers scars upon repair, vs liver that regains total function)[148,149]. It is important to note that the resolution phase is bound to happen from the onset of the inflammatory reaction[150], and comprises, like inflammation itself, five “pillars” of resolution: removal (of microbes, dead cells and cellular debris), restoration (of vascular integrity), regeneration (of tissue), remission (of fever and other inflammatory symptoms) and relief (of pain)[151].

First, pro-inflammatory cells and mediators need to be depleted and substituted by others that can induce pro-resolving environments via recruitment of non-phlogistic cells, apoptosis of neutrophils and evacuation of inflammatory cells from the tissue[152,153]. This process involves lipid mediators such as resolvins, protectins and maresins, known as specialized pro-resolving mediators (SPMs)[144,154], that can target multiple cells, including neutrophils, macrophages and monocytes, T cells, and epithelial and
endothelial cells[144] and regulate apoptosis, efferocytosis and tissue regeneration, preventing the activation of pro-inflammatory immune responses and fibrosis[144,151–156]. There is also evidence suggesting that pro-inflammatory mediators and cells play a role in the resolution of inflammation. For example, NFκB (nuclear factor kappa B) is a transcriptional factor that, during acute inflammation, activates the production of pro-inflammatory cytokines; however, it is also known to have a role in inflammation resolution (e.g. by increasing apoptosis of lymphocytes and neutrophils) [156], thus reflecting NFκB as a key regulator of immune response.

The first steps of inflammation resolution are directed towards stopping the recruitment of neutrophils and depleting the ones that had already served their purpose. Neutrophils can either undergo apoptosis, reverse migration, or lymphatic drainage[147]. Apoptosis is the main mechanism, considered the desired form of death, and it is key to establish a well-regulated inflammation resolution. Other kinds of cell death, such as autophagy, pyroptosis, necrosis, and neutrophil extracellular traps (NET) usually lead to the maintenance of inflammation and tissue damage[147].

Efferocytosis of apoptotic neutrophils is essential for a good resolution of inflammation. It is performed by phagocytic cells, especially monocytes and macrophages, which upon recognition of apoptotic neutrophils are skewed from pro-inflammatory towards an anti-inflammatory and pro-resolving status (from classical to alternate-activated macrophages)[157]. Defects in efferocytosis usually lead to a delayed inflammation resolution and cause diseases such as systemic lupus erythematosus, where patients develop autoantibodies against intracellular components of the apoptotic cells released into the tissue[158].

Tissue repair is the next critical step towards the regain of homeostasis. It is mainly directed by the immune system (neutrophils and macrophages), although fibroblasts, epithelial and endothelial cells and platelets are also needed for a successful repair[159,160]. There are numerous factors implied in resolution, such as growth factors or interleukins. Tissue repair requires proliferation and migration of epithelial cells, as well as proliferation of fibroblasts, usually through epithelial-mesenchymal transition (EMT)[161]; matrix deposition, and angiogenesis. It normally produces remodeling of
the tissue; however, there are differences regarding the state of the final repair depending on the specific organ as it has been mentioned above [148,159].

Likewise, tissue repair mechanisms rely on the type of inflammation. For example, TGF-β is critical after type-1 and type-17 inflammation; while IL-13 is responsible for tissue repair in type-2 case [149].

Recent studies propose that, at the end of inflammation, tissue does not return to previous homeostasis; but achieves an adapted homeostatic state in which, although there is a recovery of organ architecture and function, there are changes regarding cellular composition and phenotype, therefore changing the severity of future inflammatory responses[147,162,163]. This suggests that, at this state, the risk of developing chronic inflammation or autoimmune diseases outweighs the potential benefit of rapid response in case of a new infection[162].

Chronic infections from opportunistic pathogens like *Staphylococcus aureus* might happen after the achievement of this adaptative homeostasis, what could lead to the development of diseases such as asthma[164].

Inflammatory responses are needed against insults that might produce disease, such as pathogens. In normal conditions, inflammation is usually stopped on time thanks to pro-resolving and anti-inflammatory mediators and cells, which are directed from the start of the response. Both, pro-inflammatory and pro-resolving states are needed to ensure a successful regeneration of the tissue. However, it is a delicate balance that can be easily broken if any of the pieces are disturbed.

5. **EPITHELIAL BARRIER AND ALLERGIC SENSITIZATION**

An altered epithelial barrier may be critical to understand allergic sensitization. It is well known that early viral infection significantly increases the risk of developing asthma. In fact, Holt and Sly[165] stated that children suffering from viral infections in the first six months of life had an odds ratio (OR) of 4.1 of having asthma. In the same study, mite exposure was only associated with an OR 2.3. Interestingly, both risk factors combined, increased the risk of asthma to a OR of 9.0. Recently, Lopez-Rodriguez *et al*[111], have
offered some clues to understand this effect. Proteolytic activity of Der p 1 affects epithelial maturation and consolidates inflammation only if the epithelia is not well stablished, as it is the case during early viral infection. Moreover, the same authors demonstrated that high endogenous levels of glutathione S-transferase, a phenotype that has been associated with asthma, promote the enzymatic activity of Der p 1[166]. Interestingly Barber et al[167], demonstrated that Der p 1 sensitization prevalence was higher in children than in adults, while Der p 2 presented a higher overall prevalence and sIgE levels in the adult group, suggesting an early sensitization to mites and a later progression governed by Der p 2.

A second frequent respiratory sensitizer in children is Alternaria spp. The reason for this was unclear as there are other mold species naturally abundant that present a lower sensitization rate in the population. Recently Garrido-Aranda et al[168] offered some clues demonstrating that Alt a 1, the major allergen of Alternaria interacts with the epithelial SLC22A17 receptor, promoting allergic sensitization. Remarkably, Alternaria spores colonize grass death plant parts and are aerosolized together. It has been demonstrated that there are Phl p 1 like allergens in those, and that Alt a 1 and Phl p 1 interact, promoting co-sensitization, in what could represent an explanation for grass pollen sensitization evolution. Interestingly, despite being a relatively low abundant pollen protein[169], Phl p 1 is the leading grass pollen allergen sensitizer[170]. As in the case of Der p 2 in mites, Phl p 5 grass pollen allergen is usually a progression marker of grass allergy, not an early indicator of sensitization. A similar interaction of Alt a 1 has been described with thaumatins, a relevant plant allergen[171], suggesting a pivotal role of Alternaria in the onset of food allergy. Notably, in most of the cases, early sensitization to Alternaria is spontaneously resolved later in life, but it effect as “gate opener” for allergic sensitization to other sources should not be neglected[172].

Recently, it has been demonstrated that allergic sensitization to aeroallergens in the presence[139] or absence[138] of food allergy, alter the mucosal barrier, suggesting a systemic barrier defect along with allergic disease severity. This process seems to be associated to systemic signatures[173] that point to T cell proliferation[174], sphingolipids[175] and arachidonic route signaling and platelet function[176] among others. This fact might be relevant to understand allergic sensitization and disease
progression and provide a comprehensive overview of allergic diseases, where barrier function is pivotal.

**Conflict of interest**
D.B. reports grants from ALK, Allero Therapeutics, personal fees from ALK, AIMMUNE., M.M.E., E.I, J.R-C., M.D-D and C.G-C. have nothing to disclose.

**Financial sources**
This work was supported by ISCIII (PI18/01467 and PI19/00044), cofounded by FEDER “Investing in your future” for the thematic network and co-operative research centres ARADyAL RD16/0006/0015; This work was also supported by Agencia Estatal de Investigación, Ministry of Science and Innovation in Spain (PCI2018-092930) co-funded by the European program ERA HDHL - Nutrition & the Epigenome, project Dietary Intervention in Food Allergy: Microbiome, Epigenetic and Metabolomic interactions DIFAMEM. M.I.D.D. and J.R-C. and M.D-D are supported by FPI-CEU predoctoral fellowships.

**Acknowledgments**
We would like to thank Institute of Applied Molecular Medicine (IMMA, Universidad CEU San Pablo, CEU Universities, Madrid). Images where created with BioRender.com.
REFERENCES


[23] Knoop KA, McDonald KG, McCrate S, McDole JR, Newberry RD. Microbial sensing by goblet cells controls immune surveillance of luminal antigens in the colon. Mucosal Immunology. 2015;8.


[89] AnaptysBio Inc (Nasdaq: A. ANAPTYSBIO. ANAPTYSBIO Reports That the Phase 2B Clinical Trial of Etokimab in Moderate to Severe Atopic Dermatitis Does Not Meet the Primary Endpoint. 2019.


[99] Bartuzi Z, Cocco RR, Muraro A, Nowak-Węgrzyn A. Contribution of Molecular Allergen Analysis in Diagnosis of Milk Allergy. Current Allergy and Asthma Reports. 2017;17.

[100] Hashimoto T, Satoh T, Yokozeki H. Pruritus in ordinary scabies: IL-31 from macrophages induced by overexpression of thymic stromal lymphopoietin and periostin. Allergy: European Journal of Allergy and Clinical Immunology. 2019;74.


[102] Samitas K, Carter A, Kariyawasam HH, Xanthou G. Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: The one airway concept revisited. Allergy: European Journal of Allergy and Clinical Immunology. 2018;73.


to Allergen Challenge at 3 Years Among Patients With Moderate to Severe Seasonal Allergic Rhinitis. JAMA. 2017;317:615.


[164] Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? Nature Reviews Immunology. 2021;0123456789.


FIGURE LEGENDS

Figure 1. Immune protection by secretory IgA. (1) Antigen presenting cells (APCs) of the mucosa can sense bacteria antigens through pattern recognition receptors such as TLRs (toll-like receptors) and induce naïve T cell differentiation to T helper (Th) cells. (2) T cell activation can take place in special lymphatic tissue of the mucosa where Th cells promote class switch in germinal center B cells to produce IgA (IgA+B cells). (3) The IgA+B cells differentiate to plasma cells that produce dimeric IgA (Ig+ plasma cells) which becomes secretory IgA (sIgA) and will be transported to the mucus to block bacteria entry and regulate microbiota. Adapted from “IgA-mediated Gut Microbiota Regulation”, by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates.
Figure 2. Mucosal epithelial cells. In the epithelial cell barrier we can find different specialized cell types with mucosa protective functions. Here, small intestine is represented and includes: (1) Goblet cells that produce mucus and secrete antimicrobial peptides (α-MP); (2) Paneth cells that release α-MP; (3) Endocrine cells secreting neuropeptides such as serotonin, vasoactive intestinal peptide (VIP) or GABA (gamma-aminobutyric acid); (4) Tuft cells, which participate in response against helminths by stimulating innate lymphoid cells type 2 (ILC2s), which lead to intestinal eosinophilia; and (5) Microfold (M) cells, that capture luminal microbes and deliver them to dendritic cells (DCs) and macrophages (MØ) located in the lamina propria. M cells express IgA receptor (pIgR) to bind and transport secretory IgA-bound antigens (Ag). Adapted from “Intestinal Epithelium (Background)”, by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates.
Figure 3. Mucosal immune system. The classical inductive sites consist of antigen-sampling M cells, T cell areas, B cell follicles and antigen-presenting cells (APCs), which form the mucosa-associated tissue (MALT). Antigens can be captured by (1) dendritic cells (DCs) in the epithelium, by extending dendrites directly in the lumen, or (2) transported by M cells. Both cases induce DC maturation and migration to the T cell zone (3) in the MALT and (4) into draining mesenteric lymph nodes. (5) There, DCs trigger T cell activation, which depend on the nature of antigen and the local microenvironment. This results in T cell differentiation and (6) homing to effector sites. (7) Activated T cells end up in the epithelium where also intraepithelial γδ-T cells are situated. Class switching to IgA occurs in (8) mesenteric lymph nodes and in (9) MALT. (10) Then primed B cells differentiate to plasma cells that migrate to the lamina propria where produce Igs which can be secreted to the lumen. Adapted from “Intestinal Epithelium (Background)”, by BioRender.com. Retrieved from https://app.biorender.com/biorender-templates
Figure 4. Environmental factors in epithelial barrier dysfunction. Human skin and mucosa are daily exposed to environmental contaminants (cigarette smoke, particulate matter, diesel exhaust particles, ozone, nanoparticles and microplastics), airborne biological agents (dust mites, bacteria, fungi, viruses) containing toxins and allergens, cleaning products containing surfactants and detergents, and processed food containing enzymes and emulsifiers, all of which have been proven toxic for epithelial cells.
**Figure 5. Epithelial remodeling in different allergic driven diseases.** Characteristic epithelial remodeling of mucosal surfaces in atopic dermatitis (Top left, pink), eosinophilic esophagitis (top right yellow), allergic rhinitis and asthma (bottom left, green) and nasal polyps (bottom right, blue). Atopic dermatitis remodeling is characterized by dysfunction of the epithelial barrier, a reduction in skin microbiota diversity, increased allergen translocation and T cell infiltration. Eosinophilic esophagitis is characterized by barrier leakiness, epithelial to mesenchymal transdifferentiation, fibrosis and basal layer thickening. Allergic rhinitis and asthma remodeling takes place in the respiratory airways and is defined by increased extracellular matrix (ECM) deposition and fibrosis, hyperplasia of air smooth muscle (ASM) layer, barrier leakiness and mucus overproduction. Nasal polyps are characterized by increased ECM deposition and edema formation in the lumen, limited cell differentiation and tight junction (TJ) disruption, immune cell infiltration and basal layer thickening. **Legend.** ECM: extracellular matrix; ASM, airway smooth muscle layer; TJs, tight junctions.
**Figure 6. Comparison of the inflammatory response in the pro-inflammatory vs the resolution states.** Upon encountering a menace (e.g. pathogens, damage, allergens, etc.), a pro-inflammatory response ensues, starting with the recruitment of pro-inflammatory cells (1). These cells will orchestrate the immune response using inflammatory mediators (e.g. lipid mediators, such as eicosanoids; cytokines, etc), with the aim of attacking and clearing the threat (2). However, inflammatory immune response might lead to tissue damage (3), which may remain chronic if there is not a proper resolution of inflammation. Thus, after the threat is removed, the immune environment shifts towards a pro-resolving status via the recruiting of regulatory immune cells (4). These cells will ensure that homeostasis is restored using pro-resolving mediators (i.e. anti-inflammatory cytokines and SPMs lipid mediators), that will clear the inflammation site of pro-inflammatory cells both by stopping the recruitment and by activating cell death for this cells (NETs for neutrophils, apoptosis, …) (5). Cell debris will be cleaned by phagocytic cells, such as macrophages, in a process called efferocytosis (6) that prevents the reestablishment of inflammation. Finally, tissue needs to be repaired, a process that is greatly regulated by epithelial-mesenchymal transition (EMT) (7).