Epithelial Barrier Dysfunction in Type 2 Inflammatory Diseases

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Chairperson: Ignacio Dávila

Speakers: Ignacio Dávila,[1] Julien Seneschal,[2] Sven-Erik Dahlén[3]

1. University Hospital, Salamanca, Spain
2. Departments of Dermatology and Pediatric Dermatology, National Reference Center for Rare Skin Diseases, University of Bordeaux, France
3. Unit for Experimental Asthma and Allergy Research at the National Institute for Environmental Medicine (NIEM) and the Centre for Allergy Research Karolinska Institutet (CfA), Stockholm, Sweden

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Meeting Summary

This satellite symposium took place during the 49th annual meeting of the European Society for Dermatological Research (ESDR). Prof Dávila began the symposium by describing the immunology behind Type 2 inflammation as a complex interaction between environmental factors, immune response, and barrier dysfunction. He explained that the principal cells participating in innate Type 2 immunity are Type 2 innate lymphoid cells (ILC2), eosinophils, basophils, and mast cells, and that Th2 lymphocytes, dendritic cells (DC), and their main cytokines (IL-4, IL-5, and IL-13) comprise the adaptive arm of the Type 2 immune response and are essential in IgE-mediated reactions. Prof Seneschal followed by explaining that Type 2 inflammation in atopic dermatitis (AD) is a combination of immune and epidermal barrier components influenced by genetic and environmental factors. Epidermal barrier proteins are expressed in lower levels in AD, and other proteins are also dysregulated, disrupting tight junctions. Both lesional and nonlesional skin in patients with AD show

epithelial barrier dysfunction, and inflammation can lead to a vicious cycle of itching and damage. Prof Dahlén concluded the meeting by explaining that airway inflammation is one of the major factors involved in Type 2 asthma, and this can be driven by an allergic route, involving mast cells, or a nonallergic route, involving ILC2. Inflammatory cytokines also increase mucus production, one of the main causes of asthma-related death. Recent studies of asthma immunology have suggested that ILC2 are subject to feedback modulation by prostaglandin D2 (PGD2), and that both IL-4 and IL-13 are involved in hyper-responsiveness in asthmatic lung tissue.

The Immunology of Type 2 Inflammation

Professor Ignacio Dávila

Type 2 immunity evolved from a dialogue between the immune system and microbes. This form of immunity confers protection against bacteria, viruses, and parasites such as helminths, but also promotes allergic inflammation, forming the basis of AD.

Type 2 immunity involves the activation and recruitment of immune cells, and the release of inflammatory cytokines.[1] Helminths trigger the 'alarmins' IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which activate Type 2 innate lymphoid cells (ILC2). ILC2 activate and amplify other Type 2 cells such as Th2 cells and eosinophils, and release Type 2 cytokines (for example, IL-4, IL-5, and IL-13).[1]

Inflammation resulting from Type 2 immunity is a complex interaction between environmental factors, immune response (both innate and acquired), and barrier dysfunction.[2] Barrier dysfunction is a core feature of Type 2 inflammatory diseases; in asthma, respiratory viruses and allergens penetrate the epithelium causing an infiltration of immune cells and release of IL-4, IL-5, and IL-13, which disrupt bronchial epithelial tight junction proteins further.[3-5] In AD, environmental allergens pass through the dermal barrier, encouraging the infiltration of immune cells to the area, and subsequent disruption of the stratum corneum and/or tight junctions.[3]

Proteases, endotoxins, and β-glucans can destroy tight junctions, leading to the production of NF-kβ and reactive oxygen species, and the release of IL-33, IL-25, and danger signals such as uric acid.[6] These signals can act on immature DC (iDC) and ILC2 cells, causing inflammation. Some nonproteolytic allergens and genetic polymorphisms in genes such as *SPINK5* can also induce barrier defects, facilitating the penetration of allergens and giving them increased access to DC, as well as the sensitisation of Th2 cells.[6]

Early immune responses are driven by ILC2. Following activation from the epithelium, they release IL-9, IL-13, and IL-5, causing goblet-cell hyperproliferation, subepithelial fibrosis, migration of eosinophils, and subsequent TGF-β production, and smooth muscle remodelling.[6-9] ILC2 cells work through complex networks to stimulate the activation and proliferation of diverse immune cells, including macrophages, eosinophils, B cells, DC, and T cells, resulting in the production of prostaglandin D2 and other cytokines.[10] Alarmins can also activate DC, which in turn are able to capture allergens and migrate to regional lymphatic nodes. There, they present antigens to naïve T cells, inducing a Th2 response.

Eosinophils are produced in the bone marrow, and display receptors for Ig, IL, and many other cytokines. These cells can produce molecules that affect nerves, such as prostaglandins, chemokines, and growth factors.[11,12] Eosinophils also perform multiple immunomodulatory functions, including the activation of natural helper cells (NHC), macrophages, and Th2 cells, and the proliferation of T cells and neutrophils, resulting in the release of histamine and mast cell mediators.[12]

Mast cells and basophils both express receptors for IgE (high-affinity IgE receptor [FcεRI]), but priming factors differ, with mast cells primed by IL-4 and IL-6, and basophils primed by IL-3, IL-5, and IL-33. Each cell produces its own mediators, including IL-5 from mast cells, and IL-4 from basophils, as well as some common mediators, such as histamine and granzyme B.[13] The mast cell network is very important for the Type 2 immune response. It connects mast cells with nerves, where they have a bidirectional influence through TNF and PGD2, causing irritation. The

release of mast-cell granules recruits leukocytes to the endothelium, and secretion of TNF stimulates proliferation of T cells causing lymph node hypertrophy.[14] The role of basophils in the Type 2 immune response was long ignored, but peripheral basophils produce IL-4, facilitating Th2 differentiation following stimulation through the major histocompatibility complex class II. Basophils can also display the major histocompatibility complex class II molecules on their surface through trogocytosis, to stimulate Th2 differentiation.[15] Mast cells and basophils perform different roles in allergic inflammation, with basophils particularly significant in IgE-mediated chronic allergic inflammation and IgE-independent asthma.[16]

Asthma is a condition caused by Type 2 inflammation in the airways.[17] Epithelial cells release IL-25 which stimulates DC, activating Th2 cells through IL-4.[17] Th2 cells activate B cells, releasing IgE, which later occupies mast cell receptors inducing the release of histamine and cytokines, causing smooth muscle contraction.[17] Th2 cells also activate eosinophils through the release of IL-5, resulting in activation of the epithelium and consequent mucus production.[17] Some types of Th1 cells are also involved in asthma, through the TNF-α/interferon gamma (IFN-γ)-mediated activation of neutrophils.[17] The major Type 2 cytokines involved in asthma are IL-4, IL-5, and IL-13, all produced by Th2 and ILC2 cells.[18] The release of these cytokines produces a multitude of effects on the airways, including smooth muscle contraction, hyperproduction of mucus, barrier disruption, tissue remodelling, and fibrosis.[18]

AD is a result of Type 2 inflammation in the skin, involving the activation of ILC2 cells through cytokines IL-25 and IL-33, followed by itching produced by IL-31 from Th2 cells.[19] In the acute stage there is an increase in DC and Th2 activation, along with dilation of the vasculature, which leads to Th1 involvement and the release of IL-17, IL-22, and IFN-γ.[19] The major cytokines involved in AD are IL-4 and IL-13, which together result in Th2 expansion, B cell class-switching and IgE production, increased vascular adhesion and permeability, production of chemoattractants, and stimulation of the itch-scratch cycle.[20-26]

In summary, Type 2 immunity has important functions in the body, such as defence against parasites and venoms. It has three main components: the epithelial barrier, innate immunity, and acquired immunity. The principal cells participating in innate Type 2 immunity are ILC2 cells, eosinophils, basophils, and mast cells. Th2 lymphocytes, dendritic cells, and their main cytokines (IL-4, IL-5, and IL-13) comprise the adaptive arm of the Type 2 immune response and are essential in IgE-mediated reactions.

Epithelial Barrier Dysfunction and Type 2 Inflammation in Atopic Dermatitis

Professor Julien Seneschal

Healthy skin functions as a physical, permeability, and antimicrobial barrier against exogenous molecules or antigens, and maintains the internal environment.[27,28] The terminally differentiated layer of the skin, the stratum corneum, consists of corneocytes embedded in a water-repellent, lipid-rich matrix that prevents transepidermal water loss and allergen absorption.[27,29-31] Antimicrobial peptides, including β-defensin and cathelicidin, prevent colonisation and invasion by pathogenic microbes.[32] Tight junctions form a structural barrier by sealing intracellular spaces and are often disrupted in AD.[27,31,32] The plasma membrane of corneocytes is replaced by an insoluble protein structure known as the cornified cell envelope, and this forms a scaffold for lipid matrix attachment.[29,33] Filaggrin, involucrin, and loricrin are important structural proteins in the cornified cell envelope, with filaggrin binding intracellular keratin fibres, and degrading into natural moisturising factor to maintain skin hydration and low pH.[32,34-36]

The pathophysiology of AD has a multifactorial aetiology of immune and epidermal barrier components influenced by genetic and environmental factors.[37] Genetic factors can predispose individuals to AD, and include dysregulations of thymic stromal lymphopoietin (TSLP), IL-4/IL-13, toll-like receptor 2 (TLR2), IgE/FcεRI, filaggrin, serine protease inhibitor of the Kazal type (SPINK), and hornerin. Environmental factors can include allergy sensitisation, dryness, scratching, phototoxicity, and exposure to microbes or toxins which disrupt the skin barrier.

AD histology is characterised by marked spongiosis, which is vesicle formation from intraepidermal fluid, parakeratosis, the retention of nuclei in the stratum corneum, and subtle vacuolar and dermal–epidermal interface changes.[38,39] Epidermal barrier proteins such as filaggrin are expressed in lower levels in AD. Although mutations in filaggrin have been observed, they are neither necessary nor sufficient for the development of AD, suggesting that other factors are involved.[36,40] AD is associated with defects in the stratum corneum which result from filaggrin deficiency, including fewer keratohyalin granules, downregulated filaggrin degradation enzymes, dysregulated acidification pathways, and higher exposure to *Staphylococcus aureus*, herpes simplex virus Type 1 (HSV-1), and other allergens.[41] Dysregulation of claudin-1 in AD causes disruption to tight junctions, which results in the elongation of Langerhans cell dendrites into the epidermal layer, encouraging antigen sensitisation.[27]

In patients with AD, even normal-appearing skin has barrier defects, making these individuals more sensitive to environmental factors, *S. aureus,* and scratching.[8,18,19] One of the characteristics of AD is the abnormal microbiome of the skin, with increased colonisation with *S. aureus*.[42] *S. aureus* induces epithelial barrier disruption through toxin production and inflammation, resulting in increased protease activity. A recent study by Williams et al.[43] found that the introduction of *S. hominis* to the skin can inhibit *S. aureus* activity, preventing skin barrier disruption and inflammation.

The genetic expression profile in patients with AD, the 'AD transcriptome', shows that genes associated with Type 2 inflammation are upregulated in AD. However, since the transcriptome is similar in both lesional and nonlesional skin, there must also be a molecular aspect to the inflammation in lesional skin.[44] Inflammation in AD involves CD4+ and CD8+ T cells in both the dermis and epidermis, with the CD4+ cells secreting Type 2 cytokines IL-4 and IL-13, but again, this profile is similar between lesional and nonlesional skin.[45] AD is associated with increased expression of IFNγ, IL-13, and IL-22 in the skin compared to healthy individuals, and these cytokines are most abundant in lesional skin.[44] The expression levels of Type 2 cytokines IL-4, IL-5, IL-10, IL-13, and IL-31, in particular, correlate with disease activity in skin biopsies patients with AD.[46] These Type 2 cytokines have been shown to downregulate epidermal barrier proteins filaggrin and hornerin,[47] and increase TSLP production, inducing spongiosis.[48] Type 2 inflammation induces defects in the epidermal barrier, increasing skin permeability and the cutaneous innate immune response. IL-4 and IL-13 result in epidermal thickening and disturb the expression of tight junction proteins such as occluding,[49] as well as exacerbating the itch-scratch cycle by sensitising neurons to pruritogens IL-31, TSLP, and histamines.[25]

In summary, alteration of the epidermal barrier is an important feature of AD, promoting inflammation in both lesional and nonlesional skin associated with increased Type 2 proinflammatory cytokines IL-4 and IL-13. These cytokines induce defects in the epidermal barrier, leading to a vicious cycle that needs to be blocked.

Epithelial Barrier Dysfunction and Type 2 Inflammation in Asthma

Professor Sven-Erik Dahlén

It is an exciting time for asthma and respiratory disease, with many new therapeutic treatment strategies coming to market, and many more in the pipeline. In addition to new biologics, there are also new molecular targets and drug combinations, some with remarkable effects. Treatments for asthma have evolved over the last 100 years; from adrenaline, oral steroids, theophylline, and inhaled β2-agonists, through inhaled anticholinergics and steroids, long-acting drugs, and sodium cromoglycate, to anti-IgE, antileukotrienes, anti-IL-5, and anti-IL-4Rα.[50]

Asthma Pathobiology

Asthma can be described as a condition whereby airways constrict too much, too often, and too easily, resulting in impaired lung physiology and quality of life. A bronchoscopy of patient with asthma, 3 hours after a bronchoprovocation challenge, will show that although lung function may have recovered, severe inflammation and oedema remain, and the airway is still quite narrow due to smooth muscle constriction. Pathologically, asthma has four components:

airway hyper-responsiveness, airway remodelling, airway inflammation (often eosinophilic with increased fractional exhaled nitric oxide [FENO]), and bronchoconstriction.

Airway hyper-responsiveness can be measured by airway reactivity (lung function) to inhaled methacholine, or histamine. In patients with severe asthma, lung function decreases significantly after a bronchoprovocation challenge with even low doses of methacholine/histamine, and these patients can be described as 'super responders'. Current treatments for asthma are poorly effective against this problem. Airway remodelling in asthma includes epithelial dysfunction; furthermore, asthmatic airways demonstrate increasing thickness of the epithelium and reticular basement membrane, and goblet cell hyperplasia as asthma severity increases.[51] In asthmatic patients, external factors such as viruses, pollutants, and allergens are able to cross the dysfunctional epithelial barrier and trigger a Type 2 response. There are two major routes to Type 2 eosinophilic inflammation in the airway: the allergic route, which involves mast cells; and the nonallergic (innate) route, which involves ILC2 cells rarely seen in healthy lung tissue.[2] As well as eosinophilic inflammation, mucus production is stimulated by IL-13 and IL-9, and many asthma deaths are caused by mucus suffocation.[52]

Bronchoconstriction in asthma can be triggered by antigens, which bind to IgE receptors on the surface of mast cells and induce secretion of histamine, cysteinyl leukotrienes, and prostanoids, causing contraction of smooth muscle in the airway.[53] In isolated human small bronchi, challenge with anti-IgE can mimic allergen exposure and result in bronchoconstriction. This reaction can be blocked with a combination of the thromboxane receptor antagonist SQ-29,548, the H1 antagonist mepyramine, and the leukotriene synthesis inhibitor MK-886.[53] This shows the dependence on the mast cell mediators for the immediate IgE-dependent reaction.

Biomarkers of Type 2 Asthma

The main biomarkers of Type 2 inflammation in asthma are eosinophils in the blood and sputum, FENO, IgE, and periostin. Dr Morrow Brown identified the important role of eosinophils in chronic asthma in 1958, and this association has become more firmly established in the past 25 years.[54] Monitoring the eosinophilic content of sputum to guide treatment choices has been shown to reduce the severity of cumulative asthma exacerbations.[55]

FENO levels relate to eosinophilic inflammation and can be used as another marker of Type 2 inflammation.[56,57] When FENO is measured during repeated low-dose allergen challenges, it shows a clear sensitivity to steroid treatment.[58] A recent Phase III trial of dupliumab, a monoclonal antibody that inhibits IL-4 and IL-13, used FENO to predict patient response, demonstrating that patients with higher levels of FENO responded better to treatment.[59] U-BIOPRED (Unbiased BIOmarkers in PREDiction of respiratory disease outcomes) is a large, ongoing study which aims to identify novel biomarkers in severe asthma. A specialised 'breathomics' device is being used to recognise organic compounds in exhaled air of patients with severe asthma or mild/moderate asthma, and in healthy controls.[60] IgE still has a role as a biomarker of asthma, but periostin is being used less due to variable research findings.

Pivotal Role for Prostaglandin D2 in Type 2 Innate Lymphoid Cell Function

The nonallergic (innate) route to eosinophilic airway inflammation involves the stimulation of ILC2 cells.[2] ILC2 cells stimulated by alarmins such as IL-2, IL-25, IL-33, and TSLP secrete PGD2, and inhibition of PGD2 synthesis by either an nonsteroidal anti-inflammatory drugs or an experimental drug results in reduced production of IL-5 and IL-13, suggesting that endogenously produced PGD2 is necessary for ILC2 cytokine production.[61] This suggests ILC2 cells are subject to feedback modulation by PGD2 via the chemoattractant receptor-homologous molecule receptor.[63]

In summary, the pathobiology of Type 2 asthma is driven by the inflammatory cytokines IL-4, IL-5, and IL-13 that are secreted in reactions involving Th2 cells, mast cells, eosinophils, and ILC2 cells. Although Type 2 asthma responds to inhaled steroids, this is not sufficient to provide optimal control in more severe cases. Eosinophils, FENO, and total IgE are currently used to indicate Type 2 inflammation in patients with asthma, and urinary LTE4 and PGD2 metabolites show promise as novel biomarkers.

Knowledge Gaps in Type 2 Inflammatory Diseases (Panel Discussion)

Professor Ignacio Dávila, Professor Julien Seneschal, and Professor Sven-Erik Dahlén

Q: Is Th2 inflammation equally important in both asthma and atopic dermatitis, and does it occur before or after the development of barrier defects?

Prof Dahlén indicated that 30 years ago, damaged epithelium was considered to be critical in the development of asthma, but that more recently it has been considered to be more of a functional consequence of increased levels of alarmins and PGE2 rather than damage per se. Prof Seneschal said that for AD, both biological defects and inflammation are important, and promote each other. The epidermal layer is important for the production of alarmins and many other cytokines. He noted that smooth muscle contraction is one of the main symptoms in asthma but not in AD, so perhaps the response of smooth muscle to specific cytokines may explain the differences between asthma and AD. Prof Seneschal replied that in asthma, 2–3 days' incubation of airways with IL-4 and IL-13 can transform the epithelium into an asthmatic phenotype. Many cytokines are important to modify immune cells and affect smooth muscle, but the main effectors of the contraction are histamine and prostaglandins.

Q: Would restoring the epithelial barrier in atopic dermatitis be useful in the prevention of atopic dermatitis, for example, through moisturisation?

Prof Dahlén said that healthcare providers try to educate patients to apply moisturising creams. There has been some encouraging data for the use of creams in the prevention of AD in new-born babies, but in general, skin moisturisation is important to prevent new skin defects and flares in AD patients, in combination with anti-inflammatory medication.

Q: Type 2 inflammation is prominent in atopic dermatitis, but other types of inflammation are also important. In asthma, are Th2 cells more prominent than they are in atopic dermatitis?

Prof Dahlén replied that the Th2 paradigm is a huge simplification, and that severe asthma does seem to involve some neutrophilic inflammation, though it is unclear whether this is steroid-induced, or the result of bacteria. Efforts are being made through molecular phenotyping to identify new biomarkers; many different inflammatory subtypes for asthma can now be identified, but it is unclear how many are clinically relevant and can be independently targeted. Prof Seneschal said that in AD, patients could be grouped as IgE sensitive or insensitive, but the reasons for these differences can be very hard to understand.

CONCLUDING REMARKS

Professor Ignacia Dávila

In regard to the presented discussion, it would appear that although there is a Type 2 inflammatory basis to both AD and asthma, these conditions are not always associated, and do not always respond to the same treatments. The Type 2 Innovation Grant,[64] established in 2018, supports independent, novel, and innovative research projects designed to advance knowledge in the field of Type 2 immune-mediated diseases, with relevance to clinical practice. It targets not-for-profit organisations established in the European Economic Area and provides grants to research that is not related to investigational or marketed medicines. Applications are reviewed according to predefined criteria by independent international experts. Prof Seneschal is part of the expert dermatology panel established in 2019, and Prof Dahlén is on the respiratory panel. This innovation grant is proving a successful initiative, with 502 users, 252 active applications, 81 applications submitted to experts for review, and 8 selected projects thus far.

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