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REVIEW

The epithelial barrier and immunoglobulin A system in allergy

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Summary

Airway and intestinal epithelial layers represent first-line physical barriers, playing a key role in mucosal immunity. Barrier dysfunction, characterized by alterations such as disruption of cell–cell apical junctions and aberrant epithelial responses, probably constitutes early and key events for chronic immune responses to environmental antigens in the skin and in the gut. For instance, barrier dysfunction drives Th2 responses in atopic disorders or eosinophilic esophagitis. Such epithelial impairment is also a salient feature of allergic asthma and growing evidence indicates that barrier alterations probably play a driving role in this disease. IgA has been identified as the most abundant immunoglobulin in mucosa, where it acts as an active barrier through immune exclusion of inhaled or ingested antigens or pathogens. Historically, it has been thought to represent the serum factor underlying reaginic activity before IgE was discovered. Despite several studies about regulation and major functions of IgA at mucosal surfaces, its role in allergy remains largely unclear. This review aims at summarizing findings about epithelial functions and IgA biology that are relevant to allergy, and to integrate the emerging concepts and the recent developments in mucosal immunology, and how these could translate to clinical observations in allergy.

Introduction

Breathing and eating are two vital functions that carry thousands of external particles in the airway and the gastrointestinal tracts. Exposed to those inhaled and ingested particles, the mucosal surface may initiate different immunological responses. Under normal conditions, potentially dangerous antigens trigger adaptive immune responses, while innocuous antigens are eliminated without generating inflammatory responses. Mucosal immunity's prominent duty is to provide the adequate response to this continuous antigenic stimulation, to generate either inflammatory or tolerogenic immune responses, while failure in this mission could lead to recurrent infections or allergy, respectively.

Mucosal dysimmunity is thought to be responsible for the global increase in mucosal inflammatory diseases of the airway (allergic rhinitis, asthma) and gastrointestinal tract (food allergy, as well as Crohn's disease and ulcerative rectocolitis). Over the last decades, prevalence of allergic diseases has strongly increased, global asthma prevalence in developed countries reaching 9–25% in the 2000s, depending on the studies [1], and food allergy affecting today nearly 5% of adults and 8% of children [2].

On the one hand, epithelial integrity, including appropriate polarity, apical junctional complexes (AJCs) and IgA transcytosis, could play a major role in allergy, as it normally prevents allergens from adhering to the epithelium and reaching subepithelial areas. Epithelial dysfunction has been recently demonstrated to drive allergic diseases in the gastrointestinal tract and in the skin [3, 4] and is the subject of intense research in mucosal immunology. On the other hand, immunoglobulin (Ig) A represents the predominant Ig in human secretions and is widely involved in immune exclusion of antigens. The failure of the IgA system to achieve this function has been hypothesized to favour allergy, while the evidence supporting this has only been provided recently.

The present review will focus on recent advances in our understanding of the function of both mucosal barrier and humoral immune system and their relationship to allergic diseases.

The epithelium as master regulator of the mucosal barrier

The junctional barrier

The first physical barrier to inhaled or ingested antigens consists of the intestinal and airway epithelial layer, playing a key role in mucosal immunity. Epithelial apical junctional complexes (AJCs) promote cell–cell adhesion and barrier integrity, ensuring apico-basal polarity [5] and the regulation of paracellular passage of ions and macromolecules, including potential allergens. AJCs include both tight junctions and adherens junctions. Three types of transmembrane proteins compose tight junctions: (a) members of the claudin family, (b) MARVEL family members such as occludin and (c) Ig-like proteins such as junctional adhesion molecules (JAMs)[6–8]. E-cadherin and nectin family members represent the major transmembrane proteins of adherens junctions [9, 10]. Adhesive components of the AJCs are stabilized by links to intracellular proteins (zonula occludin (ZO) proteins, catenin-family proteins, actin perijunctional belt binding proteins) [11] and prevent invasion of antigens between epithelial cells (ECs). However, this junctional barrier may be altered by allergens, viruses or fungi [12, 13], cigarette smoke or air pollution, or inflammatory cytokines [14], potentially favouring IgE-mediated immunity. Increased epithelial permeability probably occurs through multiple mechanisms and results in greater penetration of inhaled particles in subepithelial areas, and subsequently elicitation of adaptive immunity as observed in allergic asthma [15, 16].

A non-exhaustive list of inducers of barrier dysfunction and their pathogenic mechanisms is summarized in Table 1.

Table 1. Mechanisms of allergen- and viral induced epithelial barrier impairment

Molecule family	Family member	Mechanism	References
House dust mite	*Der p 1*	Cleaves occluding and claudin *in vitro*	[258–261]
Pollens	*Olea europaea* *Cupressus sempervirens* *Pinus sylvestris*	Direct and indirect peptidase activity	[262]
Viruses	*Rhinovirus*	Oxidant-dependent pathway	[263–265]
	Influenza	Loss of tight junction protein claudin-4 (alveoli)	[266]
	Respiratory syncytial virus	Activation of protein kinase D	[267]
	Cocksackie	Binding to occludin	[268]
Cigarette smoke		Various pathways, including:	[269]
		- human epidermal receptor 2	[270]
		- hyaluronan	[271]
		- aquaporin-5	[272]
		- p-120-catenin	[273]
		- MUC1 glycosylation	[274]

Mechanisms of allergen-, virus- and cigarette smoke-induced increase in epithelial permeability. AJC, apical junctional complex.

Epithelial sensing and production of cytokines and alarmins

Beyond their barrier function, ECs are able to shape immune responses by secreting cytokines and 'alarmins' that regulate the adaptive immune system. Recent evidence suggests that a type 2-biased response of ECs to allergens probably contributes to the inception of several allergic disorders including asthma and atopic dermatitis [17].

Interleukin 25. Interleukin (IL)-25 is a distinct member of the IL-17 cytokine family and is also named IL-17E. Discovered in 2001[18] in highly polarized Th2 cells [19], this cytokine is constitutively expressed by several cell types [20] including ECs [21–23], IgE-activated mast cells [24], alveolar macrophages [25], eosinophils [26, 27], basophils [27], as well as endothelial cells [28] and microglial cells [29]. IL-25 is known to play a role in several inflammatory diseases such as asthma, pulmonary fibrosis and atopic dermatitis [30, 31] and is released upon exposure to proteases such as papain and trypsin, or more importantly in the context of allergy, to allergen proteases present in house dust mite (HDM) extract [32, 33]. Following binding to its receptor (IL-17RB/RA), it promotes Th2 responses and experimental asthma in mice [34]. In addition, targeting IL-25 in experimental asthma reduces Th2 cytokine production as well as airway eosinophilia and hyperresponsiveness [35, 36]. In human asthma, increased IL-25 and IL-25R mRNA levels are observed in bronchial biopsies [27], and patients with asthma expressing higher levels of epithelial IL-25 display a larger benefit (in terms of lung function) upon treatment with inhaled corticosteroids [37]. These findings underline the clinical relevance of IL-25 as a new therapeutic target in asthma [38], but the benefits of antagonizing IL-17RB/RA or directly inhibiting IL-25 require further demonstration.

Thymic stromal lymphopoietin. Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine, produced by a large variety of cells including ECs [39] and airway smooth muscle cells [40]. TSLP expression is stimulated in primary human airway epithelial and smooth muscle cells by inflammatory mediators such as IL-1β and

TNF-α, in an NF-κB-dependent manner [41, 42], by RSV [43], by cigarette smoke [44], by proteases [45], or by mechanical injury [46].

In 2002, TSLP has been demonstrated as inducing the production of Th2-attracting chemokines CCL17 and CCL22, and priming Th2-cell development by activated dendritic cells (DCs) [47], opening a new avenue in allergy pathophysiology. In asthma, expression of TSLP is increased [48] and correlates with expression of Th2-type chemokines [49]. TSLP levels are also increased in exhaled breath condensates of asthmatics [50], with increased production by their airway ECs in response to virus-derived double-strand ribonucleic acid [51, 52]. In addition, polymorphic variants of TSLP have been reported in a genomewide study of European adults with asthma [53].

In lipopolysaccharide (LPS)-primed mice, DC-derived TSLP promoted Th2 polarization following allergen sensitization [54]. Conversely, intratracheal instillation of anti-TSLP receptor antibody in asthmatic mice prevents Th2-mediated airway inflammation [55]. In ovalbumin-induced experimental asthma, TSLP is mandatory for allergic airway inflammation to develop as TSLP receptor-deficient mice show considerably attenuated disease [56], while it appeared dispensable for recall responses in established disease [57]. DCs are a first cellular target of TSLP and are primed to instruct naive T cells for Th2 polarization, while down-regulating IFN-γ and IL-10 [58]. Natural killer (NK) T cells, which seem to crucially regulate the development of allergic asthma through the production of IL-4 and IL-13 [59], also express the TSLP receptor. Incidentally, in ovalbumin-sensitized mice, TSLP overexpression induces an NK T cell-driven increase in airway hyperresponsiveness [60]. TSLP may also inhibit regulatory T cells (Tregs), leading to aberrant immune responses [61, 62].

The central role of TSLP in allergy has led to clinical programmes in allergy, providing promising results in a phase-II trial in allergic asthma with intravenous anti-TSLP monoclonal antibody [63]. The recently identified short variant of TSLP [64] and its relative expression to the full-length native form should, however, be taken into account for future targeting of this cytokine.

Granulocyte/macrophage colony-stimulating factor. Granulocyte/macrophage colony-stimulating factor (GM-CSF) is a pleiotropic cytokine that promotes the differentiation and proliferation of granulocyte and macrophage progenitor hematopoietic cells. It also regulates the survival of neutrophils, eosinophils, macrophages and DCs [65]. Since 1990, bronchial ECs are known to abundantly produce GM-CSF [66, 67], which is further enhanced by HDM allergen [68]. Overexpression of GM-CSF in mice induces spontaneous Th2 sensitization to ovalbumin [69] independently of IL-4[70], while GM-CSF$^{-/-}$ mice show a clear lack of airway eosinophils [71], antibody-driven GM-CSF neutralization preventing their sensitization to HDM [68]. Despite the ubiquitous distribution of GM-CSF, an anti-GM-CSF monoclonal antibody has been tested recently in a phase-II trial [72], to evaluate its efficacy and safety in patients with inadequately controlled asthma, but no improvement in lung function was observed in this study.

Alarmins. ECs express pattern-recognition receptors that discriminate the type of foreign agents, and following activation may release chemokines that recruit DCs, basophils and type 2 immature lymphoid cells (ILC2s) as well as damage-associated molecular patterns that promote Th2-cell mediated immunity [17]. Pathogens are recognized through pathogen-associated molecular patterns (PAMPs), while host cells may also activate this system following their activation or damage [73]. Given their intracellular source, these latter molecules first named 'endokines' are better known as 'alarmins' or 'damage-associated molecular patterns' (DAMPs). The alarmins high-mobility group box 1 (HMGB1) [74], S100 family proteins, IL-33 and IL-1α are located in the nucleus but can be released during non-programmed cell death. Their release in the subepithelial space following cell injury has been extensively studied these last years, in particular with regard to allergic diseases.

Interleukin 33. IL-33 is a member of the IL-1 cytokine family, discovered as a potent driver of Th2 polarization [75], inducing the production of IL-4, IL-5 and IL-13. Its involvement in asthma [76, 77], chronic inflammation of the gut [78] or rheumatoid arthritis [79] is well established, and increased levels of IL-33 are reported in exhaled breath condensates of asthmatics [50]. In addition, IL-33 has been proposed as an inflammatory marker of severe and refractory asthma, as its expression in bronchial biopsies positively correlates with asthma severity [80]. Furthermore, recent genetic studies in asthma identified single-nucleotide polymorphisms in the IL-33 and IL-33 receptor genes associated with asthma [53]. These results emphasize the link between allergic diseases and IL-33. IL-33 is constitutively and continuously expressed in the nucleus of ECs when they are not proliferating and displaying tight junctions, achieving inhibition of cell proliferation. Unlike during apoptosis where IL-33 is cleaved by the executor caspase-3 and caspase-7[81], IL-33 released by necrotic cells remains active and induces immune responses through binding to its IL-33 receptor, also known as ST2 receptor (ST2L). ST2L is expressed in many cell types [82] and signals through NFκB and MAPK pathways in target cells such as ILCs [83, 84],

mast cells [85, 86], macrophages [87], basophils [88] and DCs [89, 90]. The IL-33/ST2L axis is a promising target in allergic asthma, but its pleiotropic activities in several tissues and organs could represent a drawback of its therapeutic neutralization [33].

High-mobility group Box 1. Previously named amphoterin, HMGB1 is a ligand of the receptor for advanced glycation end products (RAGE) [91] and acts as an inflammatory mediator in several disorders. Two concomitant studies revealed increased sputum levels of HMGB1 in asthma [92, 93], which were inversely correlated with airway obstruction. These findings suggested its interest as putative biomarker of asthma severity [94]. In ovalbumin-induced experimental asthma, HMGB1 expression is increased, and addition of exogenous HMGB1 increased Th2 cells and levels of IL-4, IL-5, IL-6, IL-8 and IL-17 [95]. In addition, the blockade of HMGB1 binding to Gsto1 promoter region by glycyrrhizin in LPS/GalN-triggered liver-injured mice prevented apoptosis and inflammatory infiltrates [96]. In HDM-sensitized $RAGE^{-/-}$ mice, blockade of the HMGB1 downstream pathway strongly reduced Th2 responses [97]. These data strongly suggest the crucial role of HMGB1 in asthma and pave the way for therapeutic research.

Interleukin 1α. Also known as hematopoietin 1, IL-1α was described in 1985, when IL-1 was discovered to consist of two distinct proteins [98]. Its proinflammatory and profibrotic roles in the lung are well established [99], as well as its role in autoimmune diseases like rheumatoid arthritis [100, 101] or psoriasis [102]. In the past years, anti-IL-1 therapy has been a major topic of anti-inflammatory research, leading to the therapeutic anti-IL-1 receptor anakinra, the soluble decoy receptor rilonacept and anti-IL-1β antibody canakinumab [103]. A neutralizing anti-IL-1α antibody is also tested in clinical trial, with promising results [104]. The role of IL-1α in allergy remains, however, elusive, partly related to its ubiquitous location and various functions. In the gut, IL-1α has recently been identified as a key epithelial product of necrosis, amplifying and perpetuating inflammation, and is suspected to play an important role during inflammatory bowel diseases (IBDs, such as Crohn's disease and ulcerative colitis) [105]. In lung-transplanted patients, infection by *Pseudomonas aeruginosa* induces IL-1α that positively correlates with IL-8 levels and neutrophil counts, and is thought to contribute to chronic lung allograft dysfunction in bronchiolitis obliterans syndrome [106]. IL1R-lacking mice display a strongly reduced capacity to mount Th2 responses to HDM [68]. Interestingly, administration of mesenchymal stromal cells in HDM-sensitized mice reduced IL-1α and HMGB1 release in an IL-1 receptor antagonist-dependent manner, suggesting that IL-1α is a relevant target in airway allergy [107]. Furthermore, both IL-1α and IL-1β can promote tumour invasion and metastasis through inflammatory processes [108, 109], as recently reviewed [110], and anti-IL1α antibodies also represent potential new therapeutics in non-small cell lung cancer [111].

Immunoglobulin A as frontline mucosal antibodies

IgA is the predominant Ig in mucosal secretions [112], where it contributes to the frontline immune defence to inhaled and ingested antigens.

Although IgA also lies in the serum, where it predominates as monomers (5 : 1 monomeric : dimeric ratio), its main function relates to its mucosal localization where it achieves 'immune exclusion' by binding to noxious antigens and preventing adherence of microorganisms to the surface epithelium. In the airways, IgA also improves the viscoelastic properties of mucosal secretions [113]. In contrast to serum, mucosal IgA is mostly found as dimers which consist of two 160-kDa monomers of IgA covalently linked to a 15-kDa joining polypeptide (J chain)[114].

Organization of the mucosa-associated lymphoid tissues

Mucosal-associated lymphoid tissues. The mucosal immune system integrates two functionally distinct tissue compartments: inductive sites, where antigens from mucosal surfaces activate antigen-presenting cells (APCs), and subsequently naive T and B lymphocytes; and effector sites, where memory and effector B cells undergo terminal differentiation to plasma cells.

The gut-associated lymphoid tissues (GALTs) comprise the Waldeyer's ring, the Peyer's patches, the appendix and isolated lymphoid follicles [115]. In contrast, in human lung, bronchial-associated lymphoid tissues (BALT) are not constitutively present [116, 117], but develop following heavy and/or persistent/recurrent antigenic stimulation [118] as referred to as inducible BALT (iBALT) and considered as tertiary lymphoid organs. Those specialized gastrointestinal and lung structures enable local and professional antigen sampling and induction of immunity. ECs and APCs are in first line to discriminate between pathogens and harmless antigens (e.g. allergens) according to the strength of the signals first provided through two main types of pattern-recognition receptors [119] – firstly Toll-like receptors (TLRs) that may be located at the cell surface or intracellularly, depending on their subtype, and secondly (exclusively intracellular) Nod-like receptors (NLRs) [120, 121] – and to microenvironmental signals reflecting the context of the insult.

Toll-like receptors in asthma. TLRs fulfil various functions and activate nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPK) and interferon regulatory factors (IRF) signalling pathways [122]. They are involved in the maturation of DCs and regulate T cell differentiation into Th1, Th2 or regulatory T cells (Tregs). Of note, TLR5- and TLR2-activated DCs promote the differentiation into Th2 cells or Tregs by producing IL-10[122, 123], while TLR4 activation by LPS, among others, is known to recruit granulocyte, that is neutrophils, eosinophils and basophils/mast cells [122, 124].

As asthmatic inflammation is classically underlined by activation of $CD4^+$ Th2 cells, which produce IL-4, IL-5 and IL13 [125–127], several studies reported on the implication of TLRs in asthma. In Europe, a German case–control study in children identified gain-of-function single-nucleotide polymorphisms (SNPs) in the genes encoding TLR1 and TLR6 that showed protective effects in allergic asthma [128], while a Danish study established associations between asthma and SNPs in the TLR7 and TLR8 genes [129]. Moreover, the function of TLR7 was decreased in adolescents with asthma in an Australian prospective cohort study [130]. A recent meta-analysis also suggested that SNPs on TLR2, TLR4 and TLR9 genes might contribute to the development of asthma [131]. Finally, TLRs are also suspected to have immune modulating properties that can redirect allergic Th2 responses towards a more balanced Th response, notably by promoting Th1 cell activation [132]. Interestingly, several natural TLR inhibitors exist, including A20/TNFAIP3, Tollip, SOCS-1 and IRAK-M, and epithelial activation through TLR could contribute to the protective effect of farm dust exposure on asthma inception in children via the induction of A20/TNFAIP3 production [133]. In contrast, these mechanisms are deregulated during established allergic airway disease, and impaired TLR signalling could impair antiviral [134, 135] and antibacterial defence [136]. These data underscore the need for further research on TLR-driven immunomodulation in the lung, while promising results have been observed in asthma following TLR9 activation both in mice and early clinical trials [137–140].

IgA production in MALT. Antigen-specific IgA antibodies classically derive, like IgG, from conventional B cells (also called B2 cells) that have encountered their cognate antigen and have undergone somatic hypermutation. Conversely, other B cells, namely B1 cells, can secrete the so-called natural IgA or IgM antibodies, which are primarily encoded in the germ line with spontaneous antigenic specificities to naturally occurring epitopes at the surface of microorganisms such as phosphorylcholine, lysophosphatidylcholine or LPS [141]. Thus, B1 cells belong to the innate family of B cells, also including marginal zone B cells [142]. In mice, they are subdivided in B1a and B1b subsets: B1a cells express CD5, while B1b cells do not, whereas homologous subsets have not yet been described in man, possibly because of the extended expression of CD5 in various human B cells [143]. However, recent research identified two subsets of B1-like cells in human that express CD43 and share important functional features with murine B1 cells [144]. B1 cells differ from B2 cells in several regards, including location, surface markers or growth properties [145–147], as recently elegantly reviewed [148] and summarized in Table 2. B1 cells express high levels of CXCR5 and are the predominant B cell subtype in the peritoneal and pleural cavities [142], where they migrate in response to CXCL13 [149], but can also be found in the gut and airway mucosae.

Briefly, naïve B cells are primed in extrafollicular areas of BALT or GALT by $CD4^+CD40L^+$ T cells, which are activated by interdigitating APCs that have processed a luminal antigen [150]. These primed $IgD^+IgM^+CD38^+$ B cells produce an unmutated IgM that can bind the antigen with a low affinity, generating soluble immune complexes that maintain B cell memory. Mature resting B cells initially express IgD and IgM, but may undergo isotype switching to IgG, IgA or IgE when stimulated by an antigen [151] in the effector sites (lamina propria of airway mucosa). Classically, systemically administered Th1-type antigens trigger switching to murine IgG2a and Ig2b or human IgG1 and IgG3, whereas Th2 antigens promote switching to murine IgG1 and IgE, or human IgG4 and IgE [151–153]. In contrast, most of mucosal immune responses lead to switching to IgA [154] as described hereunder.

Table 2. Identification of B1 and B2 cells in the mouse, according to main phenotypic and functional features

	B1 cells	B2 cells
Surface IgA expression	High	Low
Surface B220 expression	Low	High
Surface IgD expression	Low	High
CD23 expression	No	High
Main location	Peritoneal/pleural cavities	Lymphoid follicles
Mac-1 expression	Intermediate	
CD5 expression	Intermediate in B1a subtype	
V_H repertoire	Phosphatidylcholine, Phosphorylcholine, Ig (rheumatoid factor)	High-affinity Abs to various antigenic proteins

Identification of B1 and B2 cells in the mouse, according to main phenotypic and functional features. Abs, antibodies.

Interestingly, mucosal B cells are able to switch to all Ig isotypes, according to the context.

Mucosal IgA switching and its regulation

Unlike the mouse, human IgA comprises two distinct subclasses, namely IgA_1 and IgA_2. Their constant heavy chains are encoded by distinct genes on chromosome 14 ($C\alpha_1$ and $C\alpha_2$) [155]. IgA_1 predominates in serum (as monomers), whereas IgA_2 is enriched in external secretions (mainly as dimers), representing up to 50% of total IgA [156, 157]. IgA_2 is relatively resistant to enzymatic degradation because of a 13-amino acid deletion in the hinge region, preventing the bacterial protease recognition of IgA_2 [158]. Considering that bacterial peptides bypassing the epithelial barrier may act as allergens, the relative protease resistance of IgA_2 may represent an important functional barrier at mucosal surfaces.

In activated B cells, isotype switching (i.e. class switch recombination, CSR) is initiated by activation-induced cytidine deaminase (AID) [159]. Specific DNA regions located upstream of the genes encoding the Ig heavy chain C, referred to as switch regions, undergo DNA double-strand breaks, further processed by DNA repair leading to the recombination of these regions [160]. IgA, IgG or IgE can thus be produced as the expressed C region switches from Cμ to Cα, Cγ or Cε, respectively [161], while previously rearranged Ig heavy chain variable domain confers the antigenic specificity. CSR is regulated, at least partly, by cytokines and B cell activators. Thus, IL-4 and IL-13, as prototypic Th2 cytokines, induce IgE CSR [162–164]. In the Peyer's patches and in the germinal centres of mesenteric lymph nodes, IL-21[165] and TGF-β produced by follicular T helper cells (Tfh) generate high-affinity IgA-producing plasma cells, supporting the existence of a T cell-dependent pathway for IgA CSR [166]. A T cell-independent pathway of IgA CSR also exists, at least in the gut, and generates polyreactive IgA with lower affinity [167]. More recently, retinoic acid has been shown to induce selective IgA switching in human B cells [168]. In addition, DCs can induce both T-dependent and T-independent IgA CSR through the release of IgA-inducing factors. Indeed, TGF-β1 has been demonstrated, both in human and murine B cells, to be necessary for the IgA switching [169, 170] while the release by DCs of APRIL (A Proliferation Inducing Ligand) and BAFF (B cell Activating Factor belonging to the TNF Family) has been identified as inducing IgA_2 and IgA_1 switching, respectively [171, 172]. Although IgA CSR occurs in the respiratory mucosa [173], such as following influenza infection [174], the role of lung DCs in the regulation of IgA remains unclear. Recent data, based on the recent evidence of lung microbiome [175], demonstrate that the airway microbiome regulates the ability of lung DCs to induce IgA CSR via the production of TGF-β [176].

The role of the lung and gut microbiota in allergy

Both respiratory and gastrointestinal tracts include complex communities of microorganisms, as referred to as microbiota or microbiome. In the gut, the bacterial load reaches $10^{12}/cm^3$, continuously threatening the delicate equilibrium for the mucosal integrity. In contrast, the lung has been thought sterile for long, as exemplified by the National Institutes of Health's initial Human Microbiome Project which did not include it as a site of investigation [177]. However, the recent identification of some bacterial communities in the lungs of healthy never smokers [175, 178] opened a new avenue of research in the lung.

In the gut, bacteria that penetrate the epithelial layer are usually phagocytosed by lamina propria macrophages [179], whereas invading microbes may trigger specific immune responses. Thus, after bacteria uptake by DCs and transport to inductive lymphoid sites, DCs promote activation of IgA responses at effector sites both in local and distant gut mucosal sites in order to achieve immunity or tolerance [180, 181]. In a very recent study in children, impaired IgA responses to the gut microbiota are correlated with the development of allergic diseases, including allergic asthma. These results highlight on the one hand the crucial role of IgA immunity in the prevention of allergy, and on the other hand, the strong interaction between airway and gut mucosae [182]. In the lung, changes within the microbiota (e.g. resulting from antibiotic use) have been linked to allergic airway diseases in several studies [183–185], supporting the 'microflora hypothesis' that suggests correlations between allergic airway disease, antibiotic use early in life, altered fecal microbiota and dietary changes [186]. From another perspective, absence of conventional microbiota in germ-free mice correlated with exquisite susceptibility to inflammatory bowel diseases and asthma [187]. Recently, Ruane et al. [176] showed that microbial stimuli acting on lung DCs through MyD88-dependent TLRs induce IgA class switching via the production of TGF-β, unlike lung macrophages. The study of the other factors that endow certain bacteria with the potential to induce IgA switching could reveal a fascinating matter of research.

Taken together, those results evidence the interconnection of lung- and gut-associated lymphoid tissues, as referred to the 'lung-gut crosstalk'[188]. Thus, murine lung DCs up-regulate the expression of gut-homing molecules on T cells, such as integrin $\alpha_4\beta_7$ and CCR9, allowing them to migrate to the gut and induce protection against intestinal pathogens [189]. Oral

administration of food antigens to neonatal mice provides a protection against the development of respiratory allergic diseases [190]. Finally, reovirus-primed T cells of the murine intestine confer protection to airway infection by this virus [191]. These findings further emphasize the existence of crossed mucosal responses and underline the importance of the global immune mucosal system, notably in allergy.

Transepithelial IgA transcytosis

Once secreted by mucosal plasma cells in the lamina propria, dimeric IgA (or pentameric IgM) may be transported into mucosal secretions following its transepithelial routing, which is mediated by the polymeric Ig receptor (pIgR). After IgA binding to the pIgR, which occurs owing to the expression of the J chain by these Igs, the cellular membrane invaginates into clathrin-coated vesicles that cross ECs via its intracellular membrane system and, ultimately, fuse with the apical membrane. When the pIgR reaches the apical surface of ECs, the complex with IgA (or IgM) is exocytosed after local endoproteolytic cleavage of the receptor. This cleavage releases IgA and the extracellular part of the pIgR, called secretory component (SC), which corresponds to the five extracellular Ig-like domains of the pIgR, and remains non-covalently bound to IgA. Thus, secretory (S) IgA is composed by the two monomers of IgA, the J chain and SC. Transcytosis of unbound pIgR also occurs, releasing free SC [192] that can be found in most exocrine secretions. The secretory form of IgA (and IgM) probably offers advantages (as compared to non-secretory Igs) in terms of greater stability and resistance to bacterial proteinases, as well as avidity for binding microorganisms [193].

IgA-mediated functions at mucosal surfaces

Immune exclusion. The main defence function of S-IgA is probably the binding of soluble or particulate antigens, to perform immune exclusion. Identified more than forty years ago [194], immune exclusion comprises a succession of events mediated by Igs such as agglutination, entrapment in mucus and clearance via peristalsis in the gut [195], which allows clearance of antigens before they can reach and invade tissues. Agglutination consists of the formation of macroscopic clumps of pathogens through antibody-mediated cross-linking, via polyvalent surface antigens. To which extent it does affect the bacterial growth or viral replication remains, however, unclear, with opposing results in some studies [196–198]. It has been suggested that agglutination may have various effects on pathogen functions, depending on the epitope recognized by the agglutinating antibody [199]. Mucus entrapment of pathogens by IgA has been demonstrated in both airway and gut mucosae [200, 201]. This entrapment is much greater in the presence of SC, as it associates with mucus through its oligosaccharide side chains [202, 203], further underscoring the functional superiority of S-IgA on monomeric IgA. Immune exclusion prevents the antigenic overexposure of the adaptive mucosal immune system and consequently restricts immune responses to selected antigens invading a mucosal surface. Individuals with selective IgA deficiency present with a higher incidence of allergic diseases [204, 205], probably at least partly because of the loss of this mechanism, illustrating the importance of IgA immunity to allergens.

Blockade of microbial adherence, selection of microbiota. S-IgA is also recognized for long as blocking toxins and pathogens from adhering to the mucosal epithelium [206–209], providing a protection against numerous agents such as cholera toxin or reovirus. S-IgA has been shown to block pathogen adherence by direct recognition of receptor-binding domains; of note, the recognition by specific S-IgA of the σ1 protein of reovirus, an adhesin fibre that promotes viral attachment to ECs, directly interferes with epithelial recognition and attachment [210]. More recently, it has also been shown in the gut that IgA controls the composition of the microbiome [211], which seems also the case in the lung as suggested in ageing mice with pIgR deletion [212].

Regulation of leucocytes. IgA is known to activate human eosinophils [213], through binding to the Fcα receptor (FcαRI, CD89)[214] or to an SC receptor [215] and resulting in the release of eosinophil cationic protein, eosinophil peroxidase, as well as IL-4 and IL-5. In addition, a correlation between specific IgA antibody levels and eosinophil numbers in the nasal mucosa from patients with allergic rhinitis supports this recruitment of eosinophils by allergen-specific IgA [216] *in vivo*, while soluble S-IgA is also thought to enhance eosinophil survival [217]. Degranulation of eosinophils occurs preferentially with S-IgA, but whether this relates to the presence of a C-lectin-type SC receptor is not elucidated, although free SC being also able to activate eosinophils [218]. A recent study confirmed that immune complexes of antigen-specific IgA (and IgG) may activate eosinophils, highlighting the relation between IgA responses and eosinophil activation as a key feature in several allergic diseases [219]. A recent transcriptomic study of eosinophils from patients with eosinophilic diseases (e.g. eosinophilic asthma, parasitosis, pulmonary aspergillosis and hypereosinophilic skin diseases) showed a down-regulation of transcripts involved in antigen presentation and up-regulation of

genes involved in response to non-specific stimulation, wounding and homeostasis maintenance [220], while it did not identify mucosal- or disease-specific signatures.

In addition to eosinophils, FcαRI is also expressed by other myeloid leucocytes, including neutrophils, monocytes, macrophages and DCs. The latter also express a C-type lectin called DC-SIGN/SIGNR1 (for dendritic cell's specific ICAM-3 grabbing non-integrin receptor 1) that binds both S-IgA and allergens such as HDM [221]. The ligation of FcαRI on DCs and myeloid cells could promote several protective pathways, including clearance of microorganisms that cross the epithelial barrier [222] and induction of T cell suppressive mechanisms through DLL4/Notch pathway [223] while ligation by S-IgA to DC-SIGN/SIGNR1 favours the development of IL-10-secreting tolerogenic DCs upon exposure to TLR agonists such as LPS, zymosan or CpG_{1826} [224]. These IgA-induced tolerogenic DCs promote, in turn, the expansion of regulatory T cells (Tregs), underlying a potential role for IgA in the immune homeostasis against autoimmunity.

The complex involvement of IgA in mucosal immunity is exemplified in coeliac disease, where immunity contributes both to tolerance and autoimmunity. On the one hand, IgA is a major auto-antibody as anti-endomysial and anti-transglutaminase IgA antibodies are found in most patients with coeliac disease [225] and serve as important diagnostic tools. In addition, anti-gliadin IgA antibodies represent another biomarker of the disease. On the other hand, selective IgA deficiency which represent the most common primitive immunodeficiency world-wide (prevalence 0.12–0.33%) [226] is associated with coeliac disease [227–229] with a reported prevalence increased by 10–20 fold (2.6%) in patients with coeliac disease [227]. Mechanisms underlying this observation remain unclear, but could involve defects in IgA-mediated immune exclusion of food allergens or for induction of tolerogenic DCs, as recently suggested [230]. In contrast, a recent study of the intestinal mucosa from patients with coeliac disease without IgA deficiency showed increased DC and Treg numbers [231].

The controversial role of specific IgA in allergy

The gastrointestinal tract is repeatedly exposed to dietary antigens, against which it achieves a form of oral tolerance [232]. Whereas IgG and IgA antibodies against dietary antigens is part of the normal immune response of the gut, in the PASTURE birth cohort [233], the levels of specific IgA and IgG to wheat gliadin and β-lactoglobulin in 459 1-year-old children were predictive of IgE sensitization at the age of 6. In addition, early introduction of formula milk was associated with increased β-lactoglobulin-specific IgA levels [234]. In contrast, while the effect of breast milk on barrier maturation remains controversial [235–237], the presence of soluble S-IgA in milk has been associated with a reduced risk of atopic dermatitis [238]. A beneficial role of specific, secretory IgA responses was also suggested in paediatric studies [239–241].

In allergic rhinitis, IgA increases in a biphasic manner in the nasal mucosa after allergen challenge [242], while a specific IgA response has been reported in the nasal and bronchial mucosa from patients with allergic rhinitis and/or atopic asthma sensitized to HDM [243], grass [244], ragweed [245] or birch pollen [246]. As opposed to the results of the PASTURE cohort, several studies reported that the production of allergen-specific IgA antibodies is associated with tolerance to allergens. Evidence rather supporting a protective role for IgA in allergy accumulates, as IgA deficiency represents a risk factor for allergy [247]. Interestingly, treatment of mice with the cholera toxin B, a mucosal adjuvant, suppressed the development of experimental asthma to ovalbumin and this was associated with an increased IgA response. The benefit was transferable to other mice by B (but not T) lymphocytes and was not observed in $pIgR^{-/-}$ mice, suggesting the importance of S-IgA in mucosal tolerance in this model [248]. In contrast, in models of hypersensitivity to self-antigens, the associated IgA response appeared dispensable to mount oral tolerance [249].

In a recent study, production of α-1,3-glucan-specific IgA in neonatal mice prevented the development of cockroach allergy [250], a feature potentially relevant to severe asthma [251]. HDM-specific IgA_2 was also associated with protection against eczema in allergic patients [252], while low levels of casein-specific IgA were found in children with food protein-induced allergic enterocolitis [241]. In addition, spontaneous tolerance to bee venom or cow's milk after prolonged exposure was associated with allergen-specific IgA [253, 254], and intranasal administration of ragweed-specific IgA protected against allergic inflammation in sensitized mice [255]. We have seen increases in serum allergen-specific IgA_2 following allergen immunotherapy [256], correlating with nasal mucosal expression of TGF-β, a key cytokine for mucosal tolerance and IgA synthesis [257].

The specific role of IgA and its subclasses, as well as the regulation of its production and transport in these allergic diseases, at the chronic stage, remains, however, poorly known.

Conclusion and perspectives

Mucosal immunity is influenced by multiple and complex components and aims normally to provide our mucosal surfaces with responses to antigens and microbes that are reaching these tissues of the body.

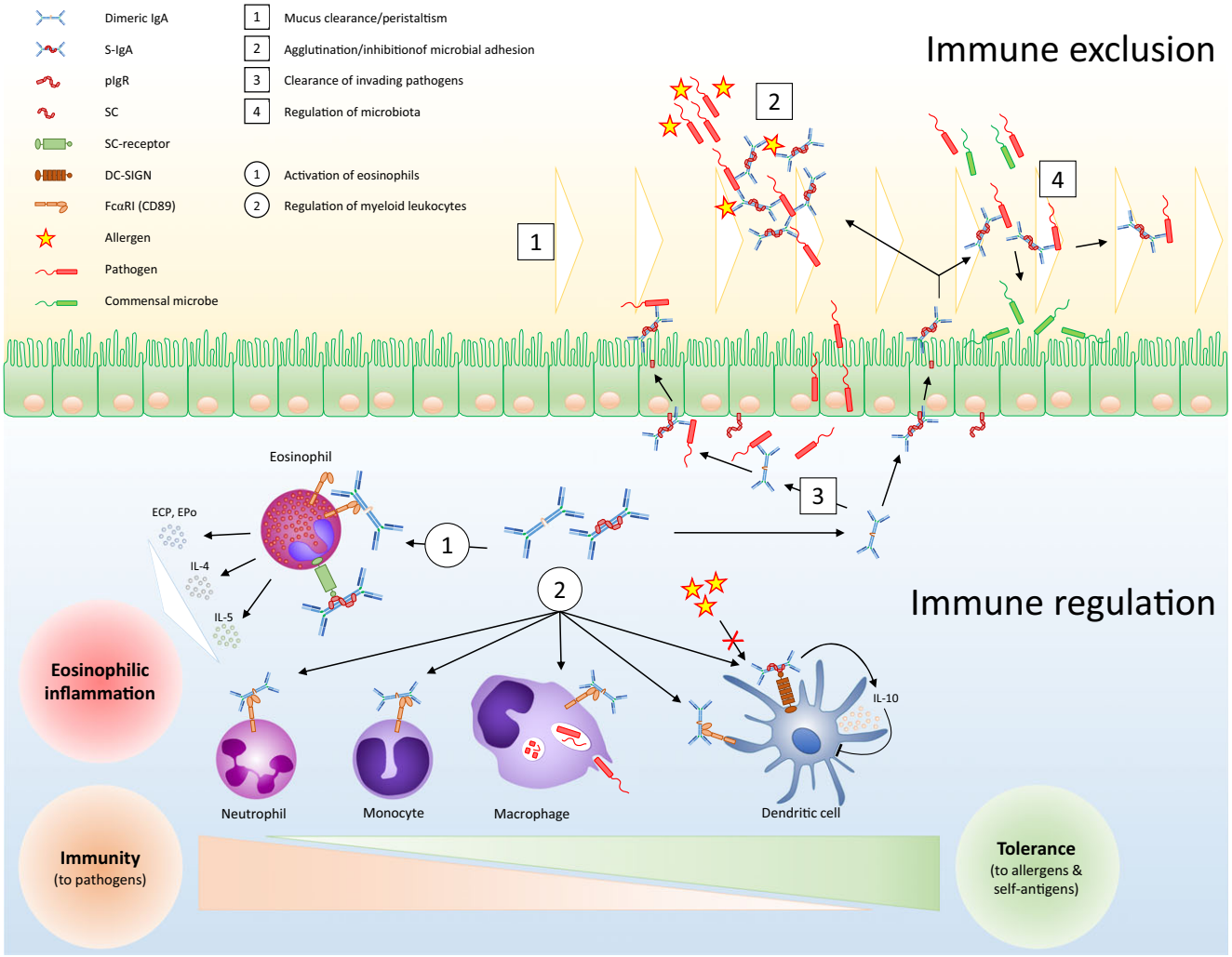

Fig. 1. Multifaceted functions of IgA in mucosa. After transcytosis through pIgR-driven routing, S-IgA performs its endoluminal duties (boxed numbers) such as immune exclusion, regulation of microbiota and neutralization of antigens plugged in mucus, while submucosal IgA may bind to various types of leucocytes (encircled numbers), leading to various outcomes (eosinophilic inflammation, adaptive immunity or immunomodulation) according to the cell type and microenvironmental signals including cytokines, costimulatory and other host factors. For example, IgA may regulate dendritic cells (DCs), which can either promote adaptive immunity or tolerance in an autocrine IL-10-dependent manner.

The epithelial barrier is critical to limit the global amount of antigens and microbes that are able to trigger the immune system, and includes mucociliary clearance, apical junctional complexes and secretion of antimicrobial peptides and secretory IgA which allows immune exclusion. Deregulation of one or several of these mechanisms may lead to increased epithelial permeability to antigens and could subsequently promote allergic or infectious disorders in the gut or the lung.

More specifically, the IgA system contributes to mucosal immunity in target organs of allergic diseases, such as airway and gut, by providing a frontline barrier for the exclusion of allergens and pathogens. IgA fits perfectly with this role, owing to its non- or poorly pro-inflammatory features. Whether IgA, which is dispensable to develop tolerance to self-antigens, is required to mount tolerance to allergens remains unclear. In human allergic diseases, IgA production has been associated with spontaneous remission of food allergy to cow's milk or anaphylaxis to bee venom, as well as with tolerance to grass pollen following allergen-specific immunotherapy, while IgA deficiency represents a risk factor for allergy. On the other hand, secretory IgA has the potential to activate eosinophils, and a specific IgA response is part of the autoimmune reactivity in coeliac disease. In addition, accumulating evidence suggests that IgA contributes to regulate the mucosal microbiome, probably both in the gut and lungs, highlighting another regulatory pathway involving IgA (Fig. 1). Further studies are clearly needed to further decipher the complex involvement of IgA in

mucosal immunity to allergens, which should integrate the interplay between allergenic, microbial and host components.

Conflict of interest

The authors declare no conflict of interest.

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