The role of airway epithelial cells and innate immune cells in chronic respiratory disease

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Abstract

An abnormal immune response to environmental agents is generally thought to be responsible for causing chronic respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Based on studies of experimental models and human subjects, there is increasing evidence that the response of the innate immune system is crucial for the development of this type of airway disease. Airway epithelial cells and innate immune cells represent key components of the pathogenesis of chronic airway disease and are emerging targets for new therapies. In this Review, we summarize the innate immune mechanisms by which airway epithelial cells and innate immune cells regulate the development of chronic respiratory diseases. We also explain how these pathways are being targeted in the clinic to treat patients with these diseases.

Chronic lower respiratory diseases most commonly manifest as asthma or chronic obstructive pulmonary disease (COPD), and they are a leading cause of morbidity and mortality throughout the world[1,2]. It is widely believed that an abnormal inflammatory response to environmental agents in genetically susceptible individuals is responsible for causing this type of disease. Environmental agents that may trigger asthma or COPD include allergens, tobacco and wood smoke, and microbial pathogens. Indeed, there has been considerable progress in defining how the immune system of the lungs responds to these agents.

The conventional view has been that the adaptive immune response is crucial for the type of long-term inflammation that is required to drive chronic respiratory disease. This scheme has been particularly well developed for allergic reactions, but has also been extrapolated to explain the immune responses that are induced by non-allergic stimuli[3]. However, an alternative view that is gaining wider acceptance is that the innate immune system also

drives chronic respiratory disease (FIG. 1). This conceptual shift raises the possibility that sentinel epithelial cells and immune cells might be essential components of pathogenesis, and might represent new targets for therapeutic intervention. A particular challenge is to explain how innate immune responses, which are traditionally viewed as being transient in nature, can drive the type of long-term immune activation that is seen in the context of chronic inflammatory disease.

In this Review, we summarize the innate immune mechanisms that regulate the development of chronic respiratory diseases, focusing on asthma and COPD. We describe the recent data that have uncovered how airway epithelial cells (AECs) and innate immune cells contribute to the pathogenesis of airway disease, and we then explain how these insights are being translated into therapeutic applications. We highlight the emerging data that suggest a role for respiratory viral infection as a key trigger for the initiation, exacerbation and progression of the immune responses that underlie chronic airway disease. Related to this, we also focus on how long-term reprogramming of AECs may account for how the innate immune system can drive the chronic activation of immune effector cells that mediates lifelong disease. For a more detailed discussion on specific aspects of the innate immune system, we refer the reader to other recent reviews[4–9]. We conclude with a perspective on how new insights into the normal and abnormal function of the innate immune system can help to explain the biological and pathological outcomes of the response to inhaled stimuli, and can provide the basis for improving treatments for one of the most common and deadly types of chronic disease in the world.

AECs in chronic respiratory disease

One of the first issues in addressing the role of the innate immune response in the development of airway disease is to define the initial responders to inhaled stimuli of disease. Airway dendritic cells (DCs) fulfil this sentinel role in the adaptive immune response, and it has been proposed that defects in DC function in response to allergen exposure or viral infection lead to asthma[10]. However, in the case of the innate immune response, the AECs of the lower respiratory tract may be the early responders that direct the subsequent immune response. This possibility first became apparent in studies showing that AECs might control immune cell infiltration into the airways during experimental asthma[11,12]. This concept has since broadened to one in which AECs monitor the external environment by using pattern recognition receptors (PRRs) to sense potentially dangerous inhaled materials[13–15]. Below, we describe how AEC-expressed PRRs can orchestrate the acute innate immune response to inhaled allergens and infectious viruses. We also introduce the fundamentally new concept that an airway progenitor epithelial cell (APEC) subset of AECs may drive a recurring innate immune response that leads to chronic respiratory disease[16].

PRRs in acute responses of AECs

Similarly to the epithelial cells in other barrier tissues, AECs express various PRRs, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs)[17]. The roles of these various PRR groups have been studied in the AEC response to both allergens and viral infections (FIG. 2), but much of the current scheme for PRR actions

(especially *in vivo*) is extrapolated from research on immune cells (especially antigen-presenting cells) and is likely to be modified with further study.

Previous work on the response to allergens has concentrated on the TLR subset of PRRs. In this case, there is evidence that TLR4 participates in the recognition of aeroallergens and thereby triggers maladaptive allergic reactions. For example, house dust mite antigen from *Dermatophagoides farinae* and fungal protease from *Aspergillus oryzae* can activate TLR4 signalling that leads to allergic responses[18,19]. Furthermore, these signals may be delivered through the activation of TLR4 on AECs. However, it still needs to be determined whether TLR4 signalling occurs primarily in AECs or in TLR4-expressing innate immune cells, such as airway macrophages, *in vivo*. Moreover, the existing analysis is largely confined to experimental models in cells and mice, so it remains uncertain whether there is similar signalling in humans. It will also be important to define the pathway from TLR4 activation to the development of a type 2 immune response (BOX 1) that is typical of allergy and to determine whether this pathway shares features of type 2 responses that might be triggered by non-allergic stimuli, such as viral infections.

Different subsets of PRRs may participate in the immune responses to the types of viruses that are thought to commonly trigger airway disease — for example, respiratory syncytial virus (RSV), human rhinovirus (HRV) and influenza A virus[20]. TLR3, TLR7, TLR8 and TLR9 can drive the response to these infections through the recognition of viral nucleic acids, primarily within intracellular endosomes. The activation of these TLRs stimulates the production of type I and type III interferons (IFNs), as well as other cytokines[21,22]. TLR3, which recognizes double-stranded RNA, seems to be especially relevant to the response of AECs to RSV[23], HRV[24,25] and influenza A virus[26]. TLR7, TLR8 and TLR9 — which are also activated by virus-associated nucleic acids[27–30] — do not seem to have a non-redundant role in the response to viral infection *in vivo*. The activation of TLR3 (as well as TLR7) generally promotes the acute inflammatory response to respiratory viruses, although TLR3 may attenuate the acute type 2 inflammation that develops after RSV infection in mice[31–37]. However, the connection between acute TLR activation and the chronic inflammation that is found in human respiratory disease remains uncertain. Nonetheless, there are ongoing efforts to develop TLR agonists, adjuvants and antagonists as therapeutics for asthma and COPD[38].

RLRs — such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) — also recognize viral nucleic acids[39–41], but interaction of these receptors with their ligands occurs within the cytoplasm. The subsequent PRR signal travels through caspase activation and recruitment domains (CARDs), as well as the adaptor mitochondrial antiviral signalling protein (MAVS; also known as IPS1, VISA and CARDIF), to induce IFN-regulatory factor 3 (IRF3) expression and subsequent IFN production[42–44]. MDA5 has been reported to sense positive-strand RNA viruses, such as HRV, whereas RIG-I recognizes negative-sense single-stranded RNA viruses, such as RSV and influenza A virus[41]. In contrast to some PRRs, the loss of MDA5 causes a marked compromise in host defence against mouse parainfluenza virus (also known as Sendai virus)[20], suggesting that RLR-dependent recognition may have a non-redundant role in host defence against respiratory viral infection. Thus, RLRs probably have a considerable

influence on the severity of infection and thereby also the likelihood of post-viral airway disease.

NLRs have more recently been recognized as a component of the epithelial response to viral infection[45,46]. For example, the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome complex[47] provides a signal for pro-caspase 1 activation and the subsequent processing and release of certain interleukin-1 (IL-1) family cytokines, including IL-1β and IL-18, that mediate paracrine signalling to neighbouring cells[48]. Initial work indicated that the NLRP3 inflammasome was an essential component of the response to viral RNA during influenza A virus infection[49]. However, a comprehensive analysis of NLRs found little evidence of a role for NLRP3 in allergen-challenge mouse models of asthma[50], so more work is needed to define the role of NLRs in chronic airway disease.

IFN induction in acute AEC responses

A major consequence of PRR activation that could influence the development of airway disease is the induction of IFN production and IFN-mediated signalling[51–54]. For the signal transduction process to be effective, type I and type III IFNs must activate Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathways and thereby induce the expression of IFN-stimulated genes (ISGs) that control viral replication[55,56]. The IFN response of AECs seems to be required for adequate defence against at least some types of respiratory viruses in experimental mouse models[57]. Shornick *et al*.[57] used the mouse parainfluenza virus model but presumably any type of respiratory virus that uses AECs as the primary host cell would fit this paradigm. In that context, the IFN response of AECs has been examined to investigate whether a defective or enhanced response might promote or attenuate chronic respiratory disease, respectively.

With regard to a possible defect in the IFN response being a contributory factor in chronic airway disease, some studies have found that compared with AECs from healthy controls, AECS from patients with asthma showed a deficiency in IFNβ and IFNλ production in response to HRV[58–60]. These observations led to a limited clinical trial of inhaled IFNβ for viral exacerbations of asthma[61]. Other studies suggested that abnormal IFN receptor signalling also contributes to the development of asthma[62], and genetic analysis has demonstrated that ISGs are associated with susceptibility to asthma[63,64]. Moreover, control of viral replication and production of IFN may also be defective in AECs from patients with COPD and cystic fibrosis[65–67]. However, many studies found no defect in IFN-dependent control of HRV or other types of respiratory viruses (notably, RSV and influenza A virus) in AECs from patients with asthma, despite using similar cell culture techniques to the studies that did report a difference[68–75]. Irrespective of whether there is defective IFN production in airway disease, it may be advantageous to enhance the IFN signalling pathway as a strategy for improving antiviral defence and preventing respiratory disease[61]. Initial work has shown that genetic strategies in mice and screening approaches in small-molecule drug discovery can enhance IFN signalling and improve control of viral infection[76–78]. However, further work is required to translate these findings into clinical practice for respiratory diseases.

AEC heterogeneity

Another key aspect of the AEC response that needs to be addressed is the possible heterogeneity within the AEC population. AECs have been shown to be heterogeneous in terms of their expression of virus entry receptors[79], and it is likely that subsets of AECs will have distinct roles in activating and orchestrating the innate immune response. The major populations of AECs (namely, ciliated, secretory, mucous and basal AECs) are specialized to carry out specific roles in mucociliary function, and in the response to allergic and infectious agents. As these populations vary in terms of their susceptibility to viral infection[74,80], they are also likely to provide different PRR-dependent signals to the immune response. Moreover, other rare cell populations that are present in the airway epithelium — for example, pulmonary neuro-endocrine cells that are sensitive to chemical stimuli — may provide additional immune signals[81]. Further definition of functional AEC subsets and their roles in the innate immune response will be needed to better understand the role of the sentinel epithelial response under healthy conditions and in the context of disease.

Chronic responses of AECs

A central issue concerning the role of AECs in airway disease is how this cell population might participate in the chronic disease process. In support of an upstream role of AECs in driving inflammatory disease, there are extensive studies showing that AECs respond to allergen challenge or viral infection by producing bioactive lipids and cytokines that control the trafficking, activation and survival of immune cells. However, it has remained difficult to understand how a relatively transient AEC response can account for the persistent activation of innate immune cells that would be required for chronic airway disease.

Recent studies of a post-viral mouse model of chronic airway disease and translation of the findings into humans have provided important new insights into this issue. This post-viral mouse model relies on infection with mouse parainfluenza virus, and a distinct aspect of the model is that the characteristic features of airway disease — namely, airway inflammation, airway hyperreactivity and excessive mucus production — develop after the virus has been cleared from the lungs and persist for at least several months thereafter[82]. Moreover, the development of airway disease requires the activation of innate immune cells, including invariant natural killer T (iNKT) cells and macrophages, that produce the IL-13 that drives airway mucus production and airway hyperreactivity[83]. Closer analysis of the mechanisms that drive ongoing immune activation in this model showed that IL-33 signalling through the IL-33 receptor subunit IL-1 receptor-like 1 (IL-1RL1) was required for the induction of IL-13 production and subsequent airway disease[16]. Moreover, increased IL-33 expression was shown to primarily occur in an inducible subset of secretory AECs, as well as in a constitutive set of surfactant protein C-expressing type 2 alveolar epithelial cells that have been shown to have a stem cell function in the lungs[84,85]. Together, these findings thereby raised the possibility that a renewable cell population was reprogrammed and expanded to drive chronic inflammatory disease.

This possibility was supported by studies showing a similar increase in IL-33 expression in airway tissue from patients with COPD, along with the expression of *MUC5AC* (which encodes mucin 5AC) and genes associated with IL-13–IL-13R signalling[16]. IL-33

expression was localized to a subset of airway basal cells and, in this case, IL-33-expressing basal cells were isolated and found to maintain an *ex vivo* programme characterized by increased progenitor cell capacity and ATP-mediated release of IL-33 (REF. 16). Given the previous work on airway basal cells as progenitor cells[86], the findings reinforce the idea that APECs might provide the upstream stimulus for chronic innate immune activation that was missing from previous schemes. Together with previous results that link immune cell production of IL-13 and IL-13 signalling to mucin gene expression[82,83,87,88], the latest results suggest a new scheme linking acute infection to chronic lung disease based on long-term epithelial progenitor cells as a renewable source of IL-33 (FIG. 3). Further work will be needed to better define the development of APECs into a specialized IL-33-expressing niche. It will also be important to define the control of IL-33 production, processing and receptor binding in disease to see if it follows the variations in gene expression, protein processing and genetic variation that may be linked to airway disease[64,89,90]. Similarly, it will be useful to define the control of IL-33 release, and its relation to other epithelial danger signals such as the levels of ATP and ATP-dependent $P2Y_2$ purinergic receptors[91–93]. It will also be useful to determine whether other AEC-derived cytokines — for example, thymic stromal lympho-poietin (TSLP) — contribute to the airway disease that is triggered by allergic stimuli. In support of this idea, treating patients with asthma with TSLP-specific blocking antibodies attenuated their response to allergen challenge[94].

This proposal for APECs as key regulators of chronic inflammatory disease may be generalizable to other environmental stimuli. Thus, several groups have identified APEC populations in the mouse and human lungs that expand in response to injury[86,95–98]. Epithelial repair pathways may remain activated following the initial injury response and may be dysregulated in the subsequent repair phase after these other forms of injury as well. For example, discrete clusters of APECs — identified in the mouse by basal cell markers — were also present for several weeks after influenza A virus infection[99], which is another potential stimulus of chronic airway disease[100]. Together with the work on the post-parainfluenza virus model of chronic airway disease, these studies suggest that viral infection may result in expansion and persistence of reprogrammed APECs that could mediate ongoing susceptibility to airway disease. The specificity required for reprogramming probably depends on the type of respiratory virus, as well as the genetic and epigenetic elements of the host response, and so future studies must address all of these issues. In addition, there is substantial evidence of a role for viral infection in the pathogenesis of both asthma and COPD, but the relative importance of epithelial reprogramming for each of these diseases will need to be defined using carefully characterized patients. Experimental and clinical evidence suggests that the combination of allergen exposure and viral infection might be fundamental to the pathogenesis of asthma, whereas the combination of smoke exposure and viral infection might be fundamental to the pathogenesis of COPD. Additional studies will also need to determine the currently uncertain influence of bacterial infection (primary or secondary to viral infection), as well that of the airway and gut microbiome, on the role of the innate immune system in airway disease.

In addition to initiating the innate immune pathway to airway disease, AECs are also an important target of this pathway. Thus, a chief end product of this pathway is IL-13, which potently stimulates AEC production of inflammatory mucus that can markedly alter the normal mucociliary clearance that is required for mucosal immunity, and that is probably responsible for much of the morbidity and mortality of chronic airway disease[101,102]. Recent work has directly linked IL-13 to airway mucus production by identifying a signalling pathway involving IL-13R, calcium-activated chloride channel regulator 1 (CLCA1) and mitogen-activated protein kinase 13 (MAPK13) that regulates *MUC5AC* gene expression in human AECs, and showing that this pathway is activated in COPD[87,88] (FIG. 3). The pathway may also be coupled to mucus fluid secretion, as CLCA1 also regulates self-cleavage and consequent calcium-activated chloride channel activity[103]. New and more potent MAPK13 inhibitors have been designed that can attenuate IL-13-induced mucus production[88], and further study of these inhibitors *in vivo* will also be useful in defining the role of airway mucus in innate immunity for host defence and airway disease.

Innate immune cells

The connection between inhaled stimuli, AECs and downstream innate immune cells is typified by the IL-33–IL-13 immune axis. Accordingly, this section concentrates on the specific types of innate immune cells — namely, NKT cells, macrophages and innate lymphoid cells (ILCs) — that are particularly implicated in this pathway to chronic airway disease. We also provide an update on how granulocytes may be involved in this disease paradigm.

NKT cells

The role of NKT cells in chronic airway disease was initially proposed for allergic asthma when iNKT cells were reported to be necessary for airway hyperreactivity in a mouse model of allergen-induced asthma and were found in increased numbers in patients at baseline[104–107]. However, subsequent studies indicated that iNKT cells were not necessary for airway inflammation in the mouse model, and that the numbers of iNKT cells in bronchoalveolar lavage fluid or endobronchial biopsies were not increased at baseline and were increased only upon allergen challenge or in the presence of severe asthma[108–111]. More recent analysis has continued to search for CD1d–presented microbial and endogenous (self) lipid antigens that might activate iNKT cells, and how T cell receptor (TCR) and cytokine signals might synergize for innate-like activation of iNKT cells[112]. One report suggests that a fungus-derived glycolipid (asperamide B from *Aspergillus fumigatus*) — which may be relevant to allergic airway disease — can activate iNKT cells in an IL-33R–dependent fashion[113], but the precise mechanism for this effect and whether it is functional in human disease remains uncertain.

NKT cell contributions to chronic airway disease have also been found in the context of viral infection. In the post-parainfluenza virus mouse model of airway disease, long-term airway hyperreactivity and excessive mucus production both depend on the activation of iNKT cells and macrophages to a type 2 pattern of cytokine production and gene expression[83]. This process seems to depend on contact between the invariant Vα14–Jα18 TCR on lung iNKT cells and the oligomorphic MHC-like protein CD1d on macrophages, as

well as amplification through IL-13–IL-13R signalling (FIG. 4). Whether iNKT cells contribute to airway disease in humans is still under study. Initial analysis suggested an increase in the frequency of iNKT cells in patients with very severe COPD that may have been missed in earlier studies of less severe disease[83]. However, as yet, no studies have assessed the level of iNKT cell activation in patients with asthma or COPD, which is likely to be more informative than counting rare cell populations. Similarly, new approaches for intravital imaging of rare cell populations will allow better definition of NKT cell behaviour in experimental models and human lung samples.

Macrophages

Similarly to other innate immune cell populations, a role for macrophages in chronic airway disease was initially identified in allergen-challenge models. For example, the house dust mite allergen Der-p2 contains ligands for TLR4 and dectin 2 that can activate macrophages, as well as other cell types, in mouse models of allergy[18,114]. Allergen-associated proteases may also activate TLR4 in a process that depends on fibrinogen cleavage, with similar consequences for the stimulation of macrophages and other cell types in the airway tissue[19]. These studies suggest that aero-allergens may rely on molecular mimicry to trigger innate immune responses, but the implications for human airway disease still need to be defined.

As introduced above, analysis of the post-parainfluenza mouse model of airway disease showed that lung macrophages are capable of regulated IL-13 and IL-13R expression, and that they can thereby promote alternative M2-type macrophage activation (in contrast to the classical activation of macrophages that might be expected for a viral stimulus)[115]. Presumably, this alternative mechanism evolved to mount a long-term innate immune response independently of $CD4^+$ T cells or $CD8^+$ T cells, and thereby enable a response to pathogens that are poorly presented by classical and divergent MHC molecules but that are adequately presented by the more conserved CD1d. However, in at least some genetic backgrounds, this type of innate immune activation can also lead to chronic airway disease. Similarly to the case for NKT cells, the level of M2 macrophage activation that occurs, and its role in asthma and COPD, still needs to be fully defined[116]. However, analysis of airway tissues from patients with COPD revealed the upregulation of IL-13, the IL-13Rα1 subunit and IL-13-dependent gene products[16,88]. Other potential markers of M2 macrophage activation — such as chitinase 1, CD163, CD206 (also known as mannose receptor 1) and matrix metalloproteinase 12 (MMP12; also known as macrophage metallo elastase) — also seem to be upregulated in patients with COPD, particularly those in the most severe stages of this disease[117–119]. Further human studies are required, but it is likely that markers of M2 macrophage differentiation that are more stable and expressed at higher levels than cytokines such as IL-13 will be more reliable to track this type of response.

Innate lymphoid cells

ILCs represent a rare, lineage-negative (LIN^-) population of lymphocytes that are classified as ILC1s, ILC2s and ILC3s on the basis of cytokine production patterns that are analogous to those seen for conventional T helper 1 (T_H1) cell, T_H2 cell and T_H17 cell subsets[120]. ILC development requires the transcription factor PLZF (also known as ZBTB16), which also mediates NKT cell development[121], suggesting a link across innate lymphocyte populations.

ILC2s also require GATA-binding protein 3 (GATA3), nuclear receptor RORα and IL-7 for their differentiation[122–125], and they are most naturally connected to the type 2 immune response that is characteristic of chronic airway disease as they produce high levels of type 2 cytokines in allergen-challenge and post-viral mouse models[122–127]. ILC2s respond to IL-25 (also known as IL-17E) or IL-33 with high levels of IL-5 and IL-13 production, and LIN⁻ ILCs expressing inducible T cell co-stimulator (ICOS) can be adoptively transferred to reconstitute IL-25- or IL-33-induced airway disease in IL-13-deficient mice[128]. In addition, adoptive transfer of ILCs into ILC-depleted recombination activating gene 1 (RAG1)-deficient mice[129], as well as mice deficient in IL-13, IL-7 or IL-33R[130–132], reconstitutes some end points of IL-13-dependent airway disease. However, it is still unclear if ILC2s are sufficient or required for this type of disease. ILC2s represent only a fraction of a percent of the lymphoid population in the lungs, so they require highly selective enrichment before they can be studied. Similarly, better tools to specifically target ILC2s while maintaining an otherwise intact immune system are still needed to further define ILC2 function.

In humans, ILC2s can be identified as LIN⁻ lymphoid cells that express CD127 (also known as IL-7Rα) and CRTH2, and that can produce IL-13 following stimulation with IL-2 in combination with IL-25 and IL-33 *in vitro*[129,133]. Human ILCs can be isolated from broncho-alveolar lavage fluid and peripheral blood samples but, similarly to in mice, they represent a very small percentage of the total lymphoid cell population and they are not increased in patients with asthma compared with healthy subjects[135]. In a survey of gut, lung and nasal tissues, ILC2s were most abundant in the polyps from patients with chronic rhinosinusitis, representing 0.1–3.6% of the total CD45⁺ cells that were recovered from these tissues[133]. Although ILC2s are rare, they may still occupy a special niche in airway immunity. Lung ILC2s are primarily found along the airways in mice and perhaps in humans[134,135], where epithelial cell-activating signals, such as IL-33, could locally drive IL-5 and IL-13 production and a consequent type 2 response. In this case, IL-13 release may be further augmented by the local release of IL-25, IL-33, TSLP, prostaglandin D2 and other factors from neighbouring innate immune cells[135]. More studies are needed to define the physiological and pathological functions of ILC2s in both the human and mouse airways.

Although ILC2s have been a major focus of ILC research, it is possible that ILC1 and ILC3 populations may also contribute to the pathogenesis of respiratory disease. The ILC1 population can include NK cells, which are probably more abundant in the lungs than ILC2s or ILC3s[136]. NK cell recruitment and activation is primarily mediated by epithelial cell release of IL-15, which increases in the nasal mucosa within a few days after HRV challenge[137]. The relevance of this response to post-viral airway disease is uncertain, but NK cell expression of NK group 2 member D (NKG2D; also known as KLRK1) and granzyme B was required for the allergic response to house dust mite extract in mice[138]. Other work showed that IL-17-producing LIN⁻THY1.2⁺ ILC3s were required for airway hyperreactivity in a mouse model of asthma that was associated with obesity[139]. The ILC3 response was driven by M1 macrophage production of IL-1β, which seems to be distinct from the association of ILC2s and M2 macrophages with preventing obesity in other mouse models[140,141]. In contrast to the rarity of ILC2 cells, LIN⁻IL-17A⁺ ILC3s were detectable in the bronchoalveolar lavage fluid from patients with asthma but the contribution of ILC3s to

airway disease still needs to be defined. A provocative hypothesis is that dietary factors may alter the overall immune response by ILC2s versus ILC3s at mucosal barriers, and therefore influence the type of chronic disease that develops[6,142].

Granulocytes

Similarly to other innate immune cell populations, granulocytes are also capable of responding to AEC cytokines to mediate chronic airway disease. For example, eosinophils are typically recruited to the airways during allergic reactions to release cytokines and granular contents with the potential to cause airway dysfunction[143]. Peripheral blood and sputum eosinophilia is often used as a biomarker of a type 2 immune response, as well as responsiveness to glucocorticoids or type 2 cytokine-blocking treatments in patients with asthma[144–146]. Similar utility is found in tracking airway eosinophilia in patients with COPD, which probably reflects the overlap between asthma and COPD[147,148]. Recent studies suggest that eosinophils are activated in response to IL-33 in a manner that depends, at least in part, on a nuclear factor-κB (NF-κB)-dependent mechanism[149], but as yet there is no experimental or clinical evidence of a functional role for eosinophils in post-viral airway disease.

Mast cells are also increased in the airway tissues of patients with asthma and COPD[150,151]. Signals such as KIT (also known as SCFR) and IL-3 allow mast cells to survive in the tissue for months to contribute to the pathogenesis of chronic airway disease[152]. In addition to their effector functions in IgE-mediated allergic asthma, mast cells also express surface receptors for IL-33 and TSLP that may be activated leading to the production of type 2 cytokines independent of IgE-mediated responses[153]. Basophils are phenotypically and functionally related to mast cells, and have also been proposed for a similar effector role in type 2 immune responses in the lungs[8]. The relative importance of each innate immune effector cell population in responding to upstream epithelial signals after viral infection and allergen challenge still needs to be defined.

Neutrophils have been implicated in the development of asthma and COPD, in addition to their role in pulmonary emphysema[154]. Indeed, an initial proposal for the sentinel role of epithelial barrier cells was based on observations that AECs could recruit neutrophils to the airways during experimental asthma induced by exposure to ozone, and that neutrophil depletion could attenuate airway inflammation and hyperreactivity under these conditions[11,155]. Similarly, neutrophils are characteristic of the early response to allergen challenge, as well as viral infection, and seem to be required for a substantial component of the post-viral airway disease that develops shortly after infection is cleared (FIG. 4). In this case, a subset of neutrophils expressing CD49d (also known as integrin α4) is connected to virus-induced type I IFN production and, in turn, to the development of IgE-IgE receptor-dependent activation of resident DCs and a downstream type 2 immune response[156,157]. These observations may explain the beneficial effect of IgE-specific monoclonal antibody therapy that might occur in viral exacerbations of asthma[158].

Neutrophils have also been linked to IL-17-driven pathology in asthma[159]. For example, overexpression of IL-17 in mouse lung tissue can lead to neutrophil accumulation, and blockade of IL-17 receptor signalling with antibodies or genetic deficiency of IL-17 and

IL-17 receptor A (IL-17RA) can decrease airway neutrophil accumulation in mouse models of allergic asthma[160,161]. However, IL-17 blockade can also cause an increase in eosinophils and type 2 cytokines[162]. Moreover, the cellular source and target of IL-17 in experimental and clinical asthma seem to be variable[163,164]. Thus, methods for identifying which patients have IL-17-dependent airway disease remains problematic and may have already led to an ineffective clinical trial in a non-stratified cohort[165]. Nonetheless, an increase in the frequency of sputum neutrophils may be characteristic of patients with severe asthma and those experiencing exacerbations of COPD[166], suggesting that these subgroups might be responsive to this type of treatment if more precision could be used to identify therapeutic strategies.

Therapeutic strategies

Given the role of AECs and innate immune cells in chronic airway disease, it is also of interest to review therapeutic strategies that might influence this immune axis in patients with asthma and COPD. A number of clinical trials have used therapeutics that target type 2 responses driven by the adaptive immune system to treat patients with asthma[167–169]. However, we now recognize that at least some of these strategies (for example, IL-13 blockade) may also attenuate chronic airway disease through actions on the innate immune system (TABLE 1). Below, we highlight the Phase II and Phase III clinical trials of therapies that modify the innate immune response that leads to chronic airway disease.

IL-5-targeting approaches

One of the best examples of an approach that was initially designed to target adaptive type 2 responses but is now known to also affect innate type 2 responses has been the development of the IL-5-specific monoclonal antibodies mepolizumab and reslizumab, and the IL-5 receptor-α (IL-5Rα)-specific monoclonal antibody benralizumab. These strategies primarily target eosinophils, which are closely linked to the development of allergic airway disease, as discussed above. Initial studies suggested that mepolizumab may decrease exacerbations in patients with refractory eosinophilic asthma[170,171]. Subsequently, a multicentre study showed a significant dose-related decrease in the levels of blood and sputum eosinophilia, and a decrease in the number of exacerbations but no significant improvement in symptoms or airflow[179]. Similarly, a study of reslizumab in patients with severe eosinophilic asthma also showed a decrease in the levels of sputum and blood eosinophils and the number of exacerbations, and a modest improvement in airflow obstruction[172]. Benralizumab offers the advantage of targeting eosinophils that may already be present in the lung tissues[173], and an initial multicentre trial showed that benralizumab decreased the number of eosinophils in the blood, sputum and airway biopsies of patients with eosinophilic asthma without serious adverse events[174]. It remains difficult to predict whether therapies targeting the IL-5 signalling pathway will be beneficial for patients with asthma, and there are currently no data for a therapeutic effect in COPD. The role of the eosinophil in the innate immune pathway to airway disease is still uncertain experimentally as well, but eosinophils may accumulate in post-viral airway disease and in COPD. Moreover, IL-5 production from innate immune cells (such as NKT cells and ILCs) may also contribute to the pathogenesis

of chronic airway disease, and thus serve as additional therapeutic targets and related biomarkers.

IL-17-targeting approaches

Innate immune responses leading to IL-17 production have also been a target for clinical trials in asthma. Brodalumab is a monoclonal antibody that is directed against IL-17RA, which is subunit that is shared among all IL-17 cytokine receptors, including the receptor for IL-25. An initial study of brodalumab in patients with inadequately controlled moderate-to-severe asthma on a stable dose of inhaled corticosteroids showed no significant improvement in airflow obstruction or symptoms[165]. Subgroup analysis showed only a nominal improvement in asthma control questionnaire scores in patients with high responsiveness to bronchodilator therapy. The relative lack of efficacy may once again highlight the need for patient stratification in these studies and in those of other targeted therapeutics. Ongoing studies of innate immune responses that lead to airway disease independent of a type 2 response will help to identify and monitor patients with IL-17-dependent types of disease.

IL-13-targeting approaches

Both the conventional T_H2 cell response in allergic airway disease and the type 2 innate immune response in post-viral airway disease lead to IL-13 production, thereby making IL-13 a therapeutic target for asthma and COPD. Characterization of IL-13-dependent gene expression has also led to the development of biomarkers to guide clinical trials of IL-13-specific monoclonal antibodies, such as lebri-kizumab and tralokinumab[145,175]. In a trial that was originally designed to examine the efficacy of lebri-kizumab in patients with poorly controlled asthma stratified by type 2 immune status, a post-hoc analysis revealed that this drug was only effective in patients with increased serum levels of periostin[145]. In a subsequent trial of tralokinumab in patients with severe asthma, subgroup analysis suggested an even greater improvement in airflow measurements in patients with high blood levels of dipeptidyl peptidase 4 (DPP4) compared to those with high blood levels of periostin[175,176]. Multicentre clinical trials are underway to validate these biomarkers, but the complex nature of the innate immune system and its role in airway disease already strongly suggests that patient stratification will be required for an effective therapeutic approach to chronic airway disease. The initial biomarkers of the patients that respond to IL-13-specific therapy (namely, periostin and DPP4) are associated with activated AECs, suggesting that this cell population is an important therapeutic target, as well as having a role in the pathogenesis of chronic airway disease. Initial studies of a TSLP-specific monoclonal antibody also showed some benefit in patients with allergic asthma[94], indicating that further studies of AEC-derived type 2-promoting cytokines (such as IL-33) may also offer a potential therapeutic approach.

Several therapeutics have also been developed to target IL-13 in combination with IL-4 by blocking the IL-4Rα chain (which is encoded by *IL4RA*) that is common to the IL-4 and IL-13 receptors. For example, dupilumab is an IL-4Rα-specific monoclonal antibody that achieved significant improvements in terms of improved airflow, fewer disease exacerbations and lower expression of several biomarkers of disease — such as CC-

chemokine ligand 17 (CCL17), CCL26, fraction of exhaled nitric oxide (FeNO) and IgE — particularly in patients with increased eosinophilia[180]. In addition, pitrakinra is a recombinant IL-4 mutein that blocks ligand binding to IL-4Rα and is capable of decreasing early-phase allergen-provoked bronchoconstriction in patients with allergic asthma[177]. Pitrakinra may be more effective in patients with polymorphisms in the *IL4RA* gene[178], suggesting that pharmacogenomics may identify subgroups that might benefit from this type of therapy. IL-4 production was not found in the post-viral model of airway disease[83], but even if IL-4 is not a feature of the type 2 innate immune response, it may still be advantageous to use combined blockade of IL-4 and IL-13 in patients who are sensitive to both allergen exposure and viral infection as triggers of airway disease.

Conclusion

It has been difficult to understand how an abnormal immune response leads to the development of chronic inflammatory disease in general, and this has also been the case for chronic respiratory diseases, such as asthma and COPD. However, ongoing progress in *in vitro* and *in vivo* models, and in corresponding human studies, suggests that the innate immune response can drive the initiation, exacerbation and progression of this type of disease in response to inhaled allergens, respiratory viruses and, most probably, other environmental stimuli. The innate immune axis seems to begin with the response of sentinel AECs that then transmit activation signals to innate immune cell populations. Recent progress provides two major insights: first, that APECs may be reprogrammed and expanded with specific cytokines (such as IL-33) to provide an ongoing susceptibility to a type 2 immune response; and second, that once activated, AECs may transmit signals to various innate immune cell populations — including NKT cells, macro phages and ILCs — that in turn produce additional cytokines (such as IL-13) to drive airway inflammation, airway hyperreactivity and excessive airway mucus production. These insights have translated into the first encouraging efforts to block these cytokine signalling pathways and treat chronic airway disease.

Future studies of this type of disease need to address ongoing and crucial questions. These include: first, the nature of epithelial PRR activation and signalling, and its relationship to the development of long-term airway disease; second, a more precise definition of the mouse and human APEC niche within the AEC population, and the basis for possible viral reprogramming of these cells; third, the relationship of the pathway from epithelial cell IL-33 to immune cell IL-13 with other environmental risk factors for chronic lung disease, such as tobacco smoking and allergen exposure, as well as underlying genetic risk factors for this type of disease; fourth, the specific nature of the immune cell populations (including NKT cells, macrophages, ILCs and granulocytes) activated in this same pathway; fifth, the molecular basis for translating these epithelial cell and immune cell signals to end-organ dysfunction, including the production and processing of IL-33, as well as the signal transduction pathways and expression of IL-33R and IL-13R; and finally, the role of neutrophils in airway disease and how it relates to the cellular sources and targets for IL-17A and/or IL-17F, and other non-type 2 forms of airway disease.

Nonetheless, ongoing research progress already indicates that environmental stimuli (especially respiratory viral infections in synergy with allergic reactions and smoke exposure) can drive innate immune responses with consequences for acute as well as chronic respiratory disease. A better definition of the role of the AEC and innate immune cell components in chronic airway disease should enable the development of new, effective therapeutics for patients.

Acknowledgments

Glossary

Airway epithelial cells (AECs)	The AECs that line the airways include ciliated, mucous, secretory and basal cell types. The AECs also line the alveoli and include type 1 and type 2 epithelial cells.
Pattern recognition receptors (PRRs)	These receptors are a key part of the initial activation step for innate immune cells. They recognize pathogen-associated molecular patterns that are associated with microbial pathogens, as well as damage-associated molecular patterns associated with cell damage.
Airway progenitor epithelial cell (APEC)	These cells are able to proliferate and then differentiate into different airway epithelial cell subsets.
Innate lymphoid cells (ILCs)	A group of cells that are similar in size and shape to lymphocytes but that do not express typical markers of T cells, B cells, natural killer (NK) cells, NKT cells or the granulocyte lineage. A current paradigm divides ILCs into three groups ILC1s that produce interferon-γ; ILC2s that produce interleukin-5 (IL-5) and IL-13; and ILC3s that produce IL-17 and/or IL-22 and are also known as ILC17s and ILC22s.
M2-type macrophage activation	An immune response that involves the alternative activation of macrophages and monocytes, and is characterized by a gene expression profile that is distinctive of stimulation by interleukin-4 (IL-4) or IL-13. There is still some uncertainty over the best markers for interferon-γ-driven classical activation of M1-type macrophages versus IL-4-and IL-13-driven alternative activation of M2-type macrophages, but the concept remains useful in mouse models of disease and is a starting point for defining macrophage responses in human disease.

Periostin	A ligand for the αVβ3 and αVβ5 integrins that supports epithelial cell migration and adhesion.
Mutein	A mutant form of a protein.

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Box 1 | Type 2 immunity

Type 2 immunity is generally characterized by an immune response that exhibits a distinct gene expression profile that, in particular, includes expression of the genes encoding interleukin-4 (IL-4), IL-5 and IL-13. Some immune stimuli drive immune responses involving T helper 2 (T_H2) cells, and these reactions are therefore known as T_H2-type responses. Other types of immune stimuli can cause a type 2 response that involves alternatively activated macrophages; these reactions are described as 'M2-type responses'. Both of these responses (T_H2-type and M2-type) have been implicated in the pathogenesis of chronic airway disease. However, in some cases, studies of patients offer a limited means by which to characterize the cellular source of cytokine production. These studies must therefore primarily rely on gene expression profiles of cytokines and/or cytokine targets to categorize the immune response. In these cases, it is most appropriate to consider these conditions to represent a type 2 immune response, rather than assigning the response to T_H2 cells, M2 macrophages or even type 2 innate lymphoid cells (ILC2s).
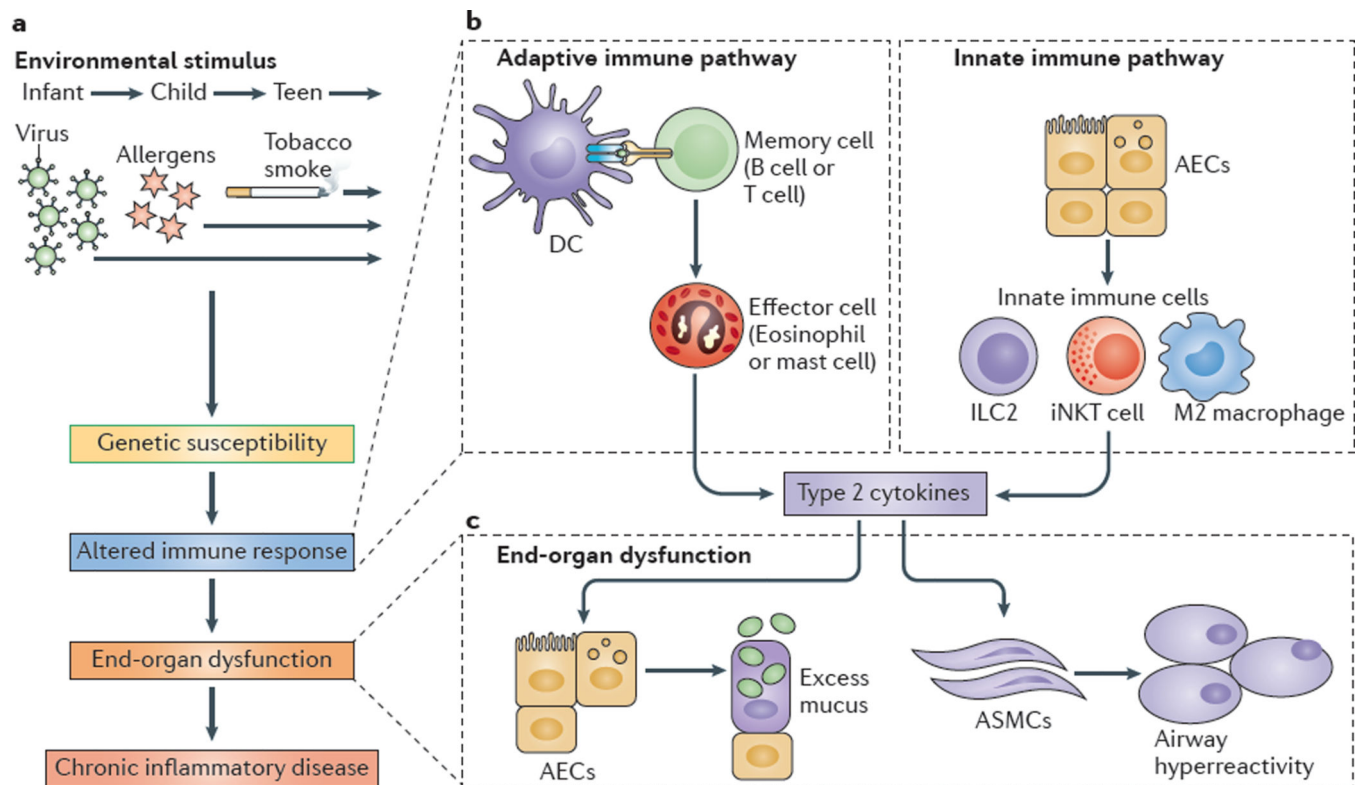
Figure 1. Adaptive and innate immune responses in chronic respiratory disease
a | Environm ental stimuli — suchas respiratory viruses, allergens and/or tobacco smoke — may act on genetically susceptible individuals to lead to an altered immune response, end-organ dysfunction and chronic inflammatory disease. **b** | An altered adaptive immune response involves antigen-presenting cells, primarily dendritic cells (DCs), that process and present antigens to memory B cells and T cells that drive the activation of effector immune cells (such as eosinophils and mast cells). Additional T cell subsets that regulate the adaptive immune response include T helper 17 (T_H17) cells, T_H9 cells and regulatory T cells (not shown). Alternatively, an altered innate immune response can involve airway epithelial cells (AECs) that activate innate immune cells, such as invariant natural killer T (iNKT) cells, M2 macrophages and innate lymphoid cells (ILCs). **c** | Effector cells or innate immune cells then produce type 2 cytokines — for example, interleukin-4 (IL-4) and IL-13 — that act on end-organ cells, especially AECs, to produce excess mucus, and on airway smooth muscle cells (ASMCs) to manifest airway hyperreactivity, which, to varying degrees, are both characteristic of patients with asthma and chronic obstructive pulmonary disease.
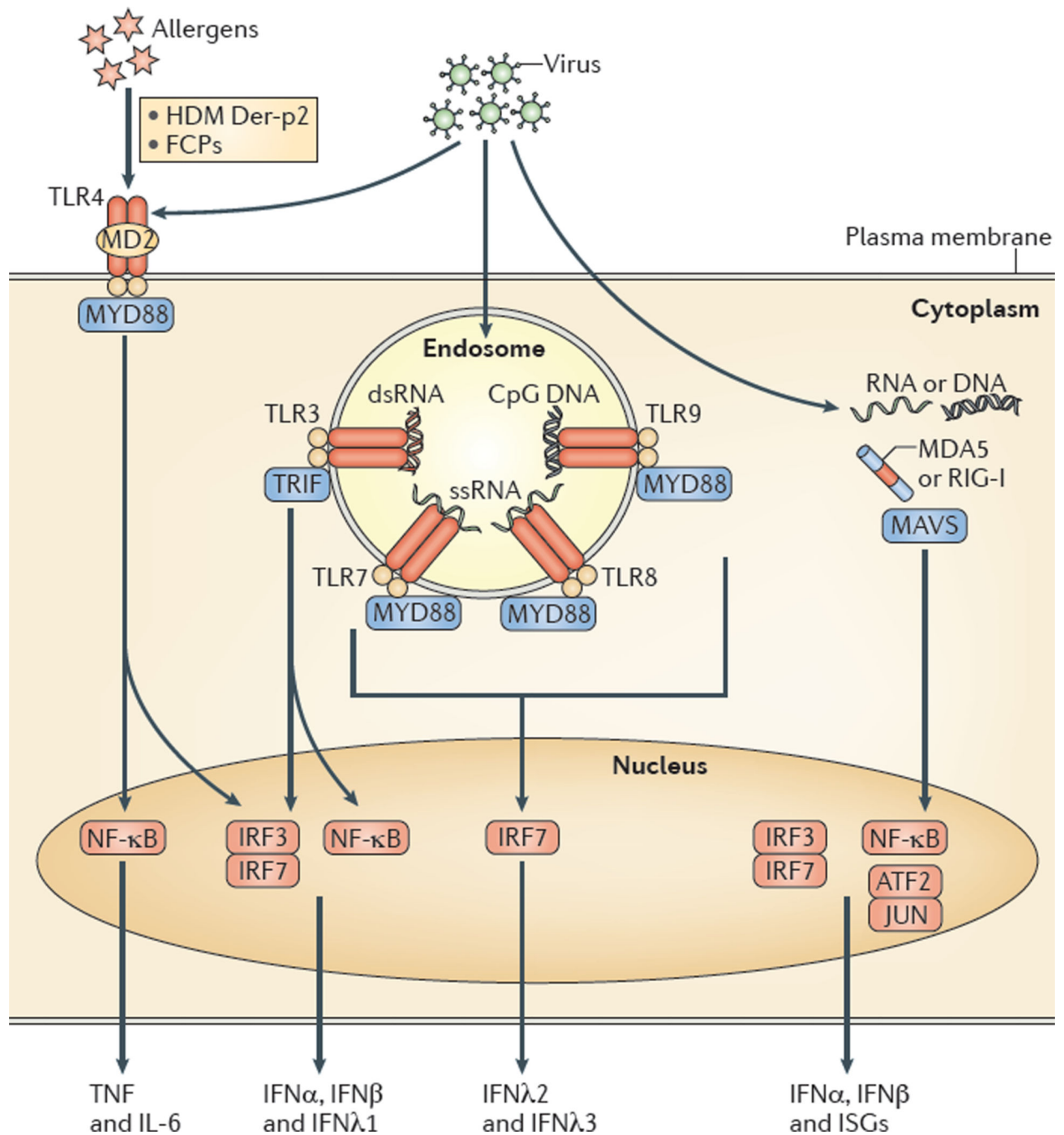
Figure 2. PRR pathways in AECs leading to airway disease
Allergens such as Der-p2 derived from the house dust mite (HDM) *Dermatophagoides farinae* and fibrinogen cleavage products (FCPs) that are generated by proteases from the fungus *Aspergillus oryzae* can act as ligands for the Toll-like receptor 4 (TLR4) complex. The activation of TLR4-dependent signalling leads to an allergic response that is characterized by type 2 cytokine production. Alternatively, viral infection can induce the activation of several additional TLRs (such as TLR3, TLR7, TLR8 and TLR9) in the endosome and can also activate RIG-I-like receptors (RLRs) — such as melanoma differ

entiation-associated protein 5 (MDA5) and retinoic acid-inducible gene I (RIG-I) — in the cytosol. In each case, activation leads to downstream signalling with the eventual stimulation of transcription factors in the nucleus and consequent expression of the indicated cytokines and interferon (IFN)-stimulated genes (ISGs). dsRNA, double-stranded RNA; IL, interleukin; IRF, IFN-regulatory factor; MAVS, mitochondrial antiviral signalling protein; MD2, myeloid differentiation factor 2; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor-κB; ssRNA, single-stranded RNA; TNF, tumour necrosis factor; TRIF, TIR domain-containing adaptor protein inducing IFNβ.
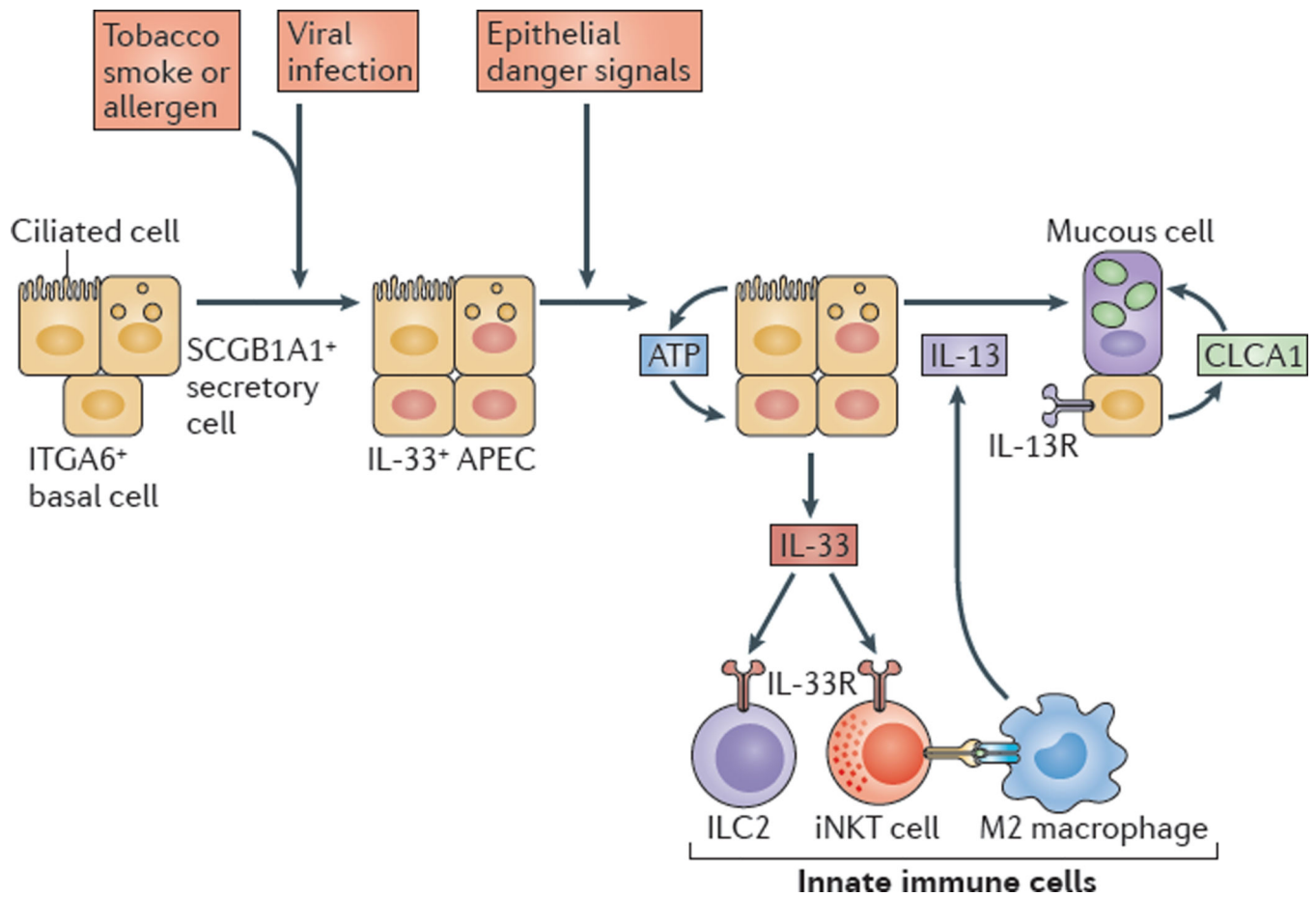
Figure 3. Innate immune responses of AECs drive airway disease

Respiratory viral infection (which is perhaps enhanced by exposure to allergens or tobacco smoke) leads to an expansion of airway progenitor epithelial cells (APECs), which are a subset of integrin α6 (ITGA6)-expressing basal cells in humans or secretory cells expressing SCGB1A1 (also known as uteroglobin) in mice that are programmed for increased interleukin-33 (IL-33) expression. Subsequent epithelial 'danger' signals stimulate ATP-regulated release of IL-33 that acts on innate immune cells in the lungs — for example, type 2 innate lymphoid cells (ILC2s) and invariant natural killer T (iNKT) cells, which can interact with M2-like macrophages to stimulate IL-13 production. IL-13 then induces IL-13 receptor (IL-13R) signalling to stimulate calcium-activated chloride channel regulator 1 (CLCA1) expression and mitogen-activated protein kinase 13 (MAPK13)-dependent signalling, which activate expression of *MUC5AC* (which encodes mucin 5AC) and, consequently, lead to airway mucous cell activation and mucus formation. Figure modified with permission from the American Society for Clinical Investigation (REF. 16).
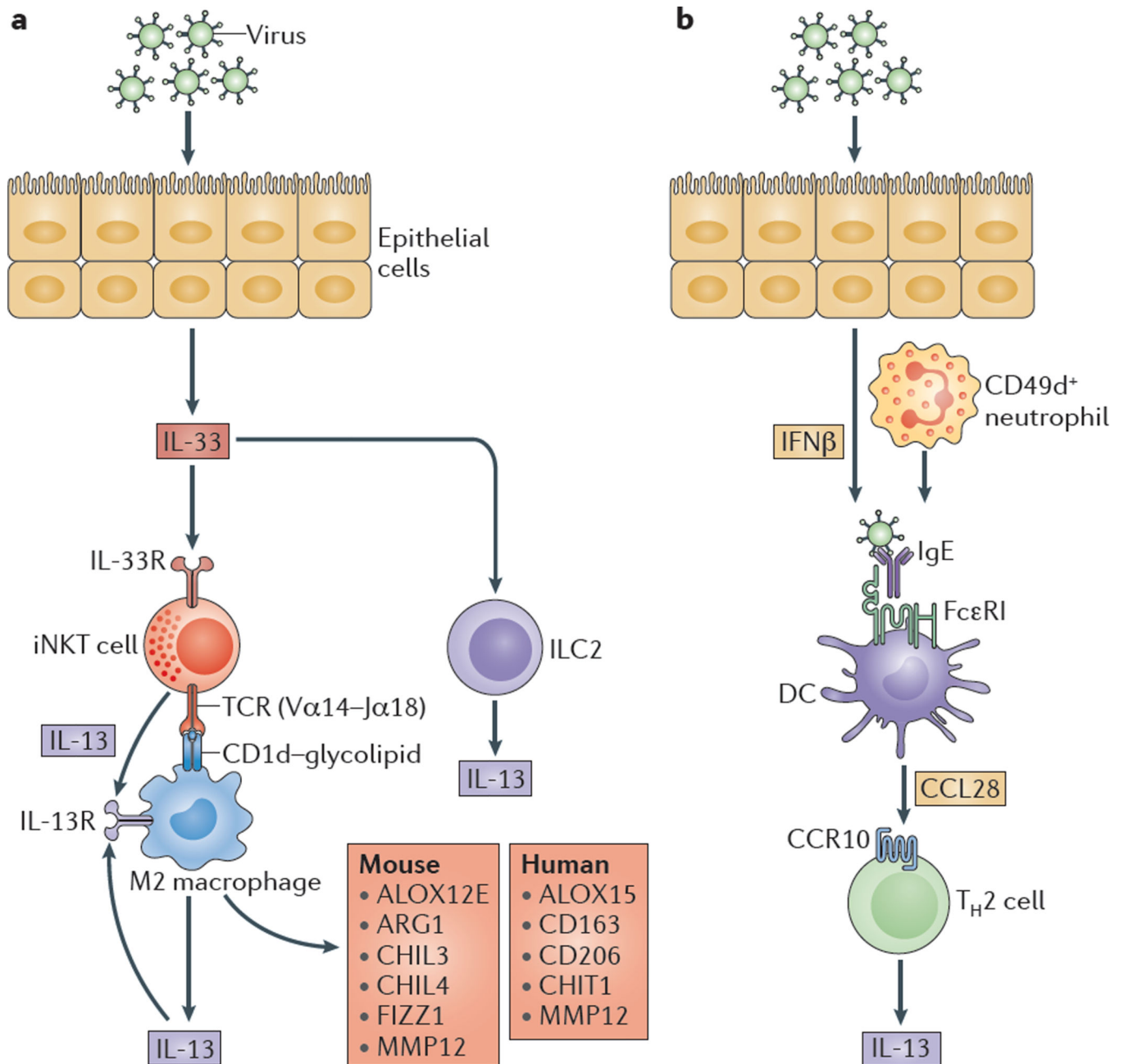
Figure 4. Innate immune cells in post-viral airway disease

a | Viral infection drives airway epithelial cell (AEC) release of interleukin-33 (IL-33) and the subse quent activation of invariant NKT (iNKT) cells that express an invariant Vα 14-Jα 18 T cell receptor (TCR) that recognizes glycolipids presented on CD1d molecules by lung monocytes and M2 macrophages. These signals lead to increased expression of the IL-13 receptor (IL-13R), and production of IL-13 that facilitates a positive feedback loop to amplify IL-13 production and alternative activation of monocytes and macrophages. Alternatively activated monocytes and macrophages are marked by epidermal arachidonate 12-lipoxygenase (ALOX12E), arginase 1 (ARG1), chitinase-like protein 3 (CHIL3), CHIL4, FIZZ1 (also known as resistin-like molecule-α) and matrix metalloproteinase 12 (MMP12)

expression in mice, and by ALOX15, CD163, CD206, chitotriosidase 1 (CHIT1) and MMP12 expression in humans. AEC release of IL-33 may also stimulate type 2 innate lymphoid cells (ILC2s), as well as effector granulocytes (such as eosinophils, mast cells and basophils; not shown), to produce IL-13. **b** | Viral infection also stimulates interferon-β (IFNβ)-dependent and CD49d^{+} neutrophil-dependent upregulation of the high-affinity Fc receptor for IgE (FcεRI) expression on resident lung dendritic cells (DCs). In turn, FcεRI activation by viral antigens and IgE leads to the production of CC-chemokine ligand 28 (CCL28) and the recruitment of CC-chemokine receptor 10 (CCR10)-expressing IL-13-producing T helper 2 (T_H2) cells to the lungs.

Table 1

Cytokine-directed therapeutics in advanced clinical trials for chronic airway disease[*]

Biologic	Mechanism of action	Efficacy in asthma	Efficacy in COPD	Biomarker for treatment
Mepolizumab	IL-5-specific monoclonal antibody	Decreased blood and sputum eosinophilia, and decreased exacerbations	Trials underway[‡]	Blood eosinophilia
Reslizumab	IL-5-specific monoclonal antibody	Decreased sputum and blood eosinophilia, and improved airway function	No trials	Sputum eosinophilia
Benralizumab	IL-5Rα-specific monoclonal antibody	Decreased blood, sputum, tissue and bone marrow eosinophilia	Trials underway[‡]	Sputum eosinophilia
Lebrikizumab	IL-13-specific monoclonal antibody	Improved airflow	No trials	Serum periostin
Tralokinumab	IL-13-specific monoclonal antibody	Improved airflow	No trials	Serum dipeptidyl peptidase 4 and periostin
Dupilumab	IL-4Rα-specific monoclonal antibody	Improved airflow, decreased exacerbations and decreased T_H2 cell markers	No trials	Blood and/or sputum eosinophilia
AMG-317	IL-4Rα-specific monoclonal antibody	Decreased exacerbations	No trials	Not reported
Pitrakinra	IL-4 mutein targeting IL-4Rα	Reduced early asthma response and decreased exacerbations	No trials	Atopy, *IL4RA* polymorphism and blood eosinophilia
Brodalumab	IL-17RA-specific monoclonal antibody	Marginal airflow improvement in patients with asthma who have a highly reversible FEV1	No trials	Not reported
AMG-157	TSLP-specific monoclonal antibody	Reduced early and late asthma response, and decreased sputum and blood eosinophilia	No trials	Atopy

COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume 1; IL, interleukin; T_H2, T helper 2; TSLP, thymic stromal lymphopoietin.

[*] Based on data from randomized, placebo-controlled trials.

[‡] Based on information from the ClinicalTrials.gov website and/or the European Union Clinical Trials Register website. Information accurate as of August 2014.