Control of lung defence by mucins and macrophages: ancient defence mechanisms with modern functions

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ABSTRACT Owing to the need to balance the requirement for efficient respiration in the face of tremendous levels of exposure to endogenous and environmental challenges, it is crucial for the lungs to maintain a sustainable defence that minimises damage caused by this exposure and the detrimental effects of inflammation to delicate gas exchange surfaces. Accordingly, epithelial and macrophage defences constitute essential first and second lines of protection that prevent the accumulation of potentially harmful agents in the lungs, and under homeostatic conditions do so effectively without inducing inflammation. Though epithelial and macrophage-mediated defences are seemingly distinct, recent data show that they are linked through their shared reliance on airway mucins, in particular the polymeric mucin MUC5B. This review highlights our understanding of novel mechanisms that link mucus and macrophage defences. We discuss the roles of phagocytosis and the effects of factors contained within mucus on phagocytosis, as well as newly identified roles for mucin glycoproteins in the direct regulation of leukocyte functions. The emergence of this nascent field of glycoimmunobiology sets forth a new paradigm for considering how homeostasis is maintained under healthy conditions and how it is restored in disease.

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Introduction

The principal function of the lungs is gas exchange. To this end, under normal tidal breathing, 8000–12 000 L of air pass through the lungs each day. Gas flows through multiple generations of conducting airways, which ultimately terminate in the alveoli. Alveoli are bounded by type I epithelial cells that cover over 95% of the lung surface. To allow for efficient exchange of O_2 and CO_2, type I epithelia are extremely thin and together with alveolar capillaries create a diffusion distance of <1 μm. Consequently, these thin surfaces are protected by elaborate defence mechanisms that must trap and eliminate particulates and pathogens before they reach the alveolar walls, while simultaneously preventing and/or suppressing potentially inflammatory responses that could injure delicate gas exchange structures. This review concentrates on the mucociliary escalator and alveolar macrophages (AMs) as crucial first and second lines of host defence in the lungs.

Airway tissues are exposed to ~100 billion inhaled particles daily [1]. Airborne particles can arise from natural and manmade sources, can vary in size and chemical composition, can differ in concentrations based on geography and local environments, and can thus result in heterogeneous pathological responses [2–8]. Most inspired materials are large enough to impact upon nasopharyngeal and tracheal mucosae where they are transported proximally by mucociliary clearance (MCC) and are ultimately eliminated by expectoration or swallowing. The remainder deposit in the lung periphery where they are ingested by AMs. Under healthy conditions, particulate deposition in the periphery is primarily limited to small particles (<1 μm diameter). However, under conditions where particulate concentrations are high or in pathological settings where MCC is impaired, larger particles can also accumulate in the lung periphery. Together, the coordinated functions of MCC and AMs eliminate inhaled particulates from the alveoli and airways, and hence comprise robust mechanisms for exogenous clearance. At the same time, clearance also removes endogenous materials that are generated during normal cell turnover or as a consequence of disease. Critically, although AM and MCC functions are ordinarily considered distinct, emerging data show that their functions are tightly linked through physiological and biochemical mechanisms. Below we describe mucus and macrophages separately, and this is followed by a discussion of emerging knowledge of interactions between them.

The mucus barrier and mucociliary clearance

MCC involves the coordinated activities of secretory cells that release polymeric mucin glycoproteins, and multiciliated cells whose apically localised motile cilia provide a means for transport and elimination. Cilia are molecular machines whose structural and motile components are highly regulated; their complex assembly, function and dysfunction in diseases are reviewed elsewhere [9, 10]. For the purposes of this review, we consider physiological roles of motile cilia, and we highlight key aspects of mucociliary interactions that are essential in the airways. MCC requires the coordinated regulation of airway surface liquid to control the osmolarity, viscoelasticity and resultant transportability of secreted mucus [11, 12]. This control is driven by electrolyte transport machinery intracellularly as well as the presence of osmolytes in the extracellular space. Although ciliated and mucous layers have been considered as separate entities ("sol" and "gel" phases), this distinction is challenged by recent studies demonstrating these "layers" as a more continuous glycoprotein hydrogel. Membrane mucins (MUC1, MUC4 and MUC16) that are present along cilia surfaces form a hydrated brush that allows for the free movement of cilia. The overlying, viscoelastic mucus layer is positioned atop this grafted brush of cilia. As a result, airway surface hydration regulates the balance between cilia and mucus structures maintained in a "gel-on-brush" conformation that promotes effective motility and MCC [13].

Loss of MCC is a significant cause of respiratory infections. For instance, impaired MCC is a primary pathophysiological feature of infection-related diseases such as primary ciliary dyskinesia (PCD), where cilia motility is impaired or absent, and cystic fibrosis (CF), where airway surface dehydration causes mucus adhesion to airway surfaces and hyperosmotic collapse of underlying cilia. Less appreciated perhaps are findings in chronic obstructive pulmonary disease (COPD) and asthma, which also show significant MCC impairment [14–21]. Unlike the primary roles of altered mucus and ciliary structures in CF and PCD, COPD- and asthma-related changes are secondary to inflammatory or injurious stimuli that cause impairments in ciliary motility and the dysregulated production of the two major secreted mucins MUC5AC and MUC5B [22–25].

Expression of the airway mucins MUC5AC and MUC5B

Under healthy conditions, MUC5AC and MUC5B are both produced in the lungs. MUC5AC is found predominantly in surface epithelia throughout the central conducting airways, whereas MUC5B is found mainly in submucosal glands of central airways (trachea and bronchi) and in non-ciliated surface epithelial cells of peripheral airways. MUC5AC levels increase in both airway surface and glandular epithelia in asthma [22, 23] and COPD [24, 26, 27]. By contrast, MUC5B levels are more variable. For example, in patients with established CF and COPD, MUC5B levels are increased in sputum [28, 29], which is predominated by central airway secretions supplied by tracheobronchial submucosal glands. However, in

patients with early or preclinical COPD or with strong allergic asthma, MUC5B levels actually decrease, especially within epithelial cells that line central and peripheral airway surfaces where *MUC5B* transcript levels are reduced by 90% or more [22–24, 27]. It is thus plausible that differential repression of MUC5B could affect MCC and contribute to lung pathologies. Indeed, recent studies in mice provide mechanistic support for this.

In mice, deletion of the *Muc5b* gene caused severe upper and lower airway MCC impairments and led to the development of lethal spontaneous infections [30]. Interestingly, although chronic infection and inflammation were prominent outcomes in *Muc5b* knockout mice, their pathobiological impacts were stronger than those observed in models of PCD. In cilia-defective *Dnaic*, *Pcdip1*, *Spef2* and *Cby* knockout mice, although MCC is severely impaired, upper airway pathologies were not reported to be lethal, and they did not carry over to the lower respiratory tract [31–33]. Thus, among MCC components in the lungs, Muc5b is a dominant regulator of homeostatic microbial elimination. In addition, during chronic spontaneous and acute experimental infections, Muc5ac production increased in *Muc5b* knockout mice. Although not entirely protective itself, Muc5ac could have played a role in delaying the effects of infections [30]. Possible explanations for differences between Muc5ac and Muc5b functions could reflect differences in their polymeric structures, glycosylation, and interactions with microbes or antimicrobial molecules. Determination of the specific and overlapping roles of Muc5ac and Muc5b remains an area of urgent investigation.

Mucin expression

MUC5AC/Muc5ac and *MUC5B/Muc5b* gene expression levels are regulated by endogenous and environmental factors. For human *MUC5B*, single nucleotide polymorphisms have been shown to regulate expression *via* control of promoter activity [34–36]. These genetic controls likely impact (or are impacted upon by) numerous innate and adaptive immune cytokine signalling pathways, as well as growth factor-regulated mechanisms that are associated with responses to inflammation, injury and tissue repair. These are reviewed extensively elsewhere [37–42]. Lastly, endogenous factors include developmental [43–46] and epigenetic [47–49] regulatory mechanisms, which can play roles in the expression of mucins in cancers.

Mucin polymerisation

The abilities of secreted mucins to regulate MCC are largely dependent on their polymer structures formed through disulfide bonds (figure 1). Like other members of the secreted polymeric mucin family, Muc5ac and Muc5b are composed of ∼5–6% cysteines (∼250–300 per molecule). They have cysteine-rich N- and C-terminal von Willebrand factor (vWF) type D-like and C-terminal cysteine knot disulfide bonding domains that are critical for intermolecular mucin assembly [51–53]. Additional highly conserved cysteine-rich CysD domains are interspersed in varying numbers in polymeric mucin carbohydrate-rich repeats [54–56]. Through intramolecular disulfide linkages, CysD domains are proposed to form hydrophobic loop structures that facilitate mucin alignment and regulate mucus mesh spacing [57]. Furthermore, in each mucin at least 100 cysteines exist that are not found in defined "domains". The majority of disulfide bonds are thought to form intracellularly during assembly. In the extracellular environment, free cysteines that do exist can become oxidised and form additional cross links that increase the elastic moduli of mucus gels [50]. Disruption of N- and C-terminal bonds or CysDs can be sufficient to "loosen" obstructive mucus. Accordingly, current mucolytic therapies such as N-acetylcysteine, as well as investigative therapies, target these by reducing disulfides and decreasing mucus viscoelasticity, thereby enhancing mucus transport [58–60]. A current challenge is to determine which therapies can be given at doses that are well-tolerated and still maintain the benefits of efficient defence.

Mucin glycosylation

Whereas disulfide polymerisation is an important but underappreciated aspect of secreted mucins, their glycosylation is perhaps more eminent. Mucins are defined by their heavy glycosylation, especially within variable-sized glycan-rich domains (figure 1). In MUC5AC and MUC5B, these regions are called "PTS" domains due to their enrichment in prolines, threonines and serines. PTS-rich repeats are sites of O-glycosylation, starting with N-acetylgalactosamine on serine and threonine residues. Galactose and N-acetylglucosamine are then attached and elaborated linearly or in branches, and the sugars can be modified by sulfation or by the addition of terminal sialic acid and fucose glycans. Two chief purposes of mucin glycans are to adsorb water and to participate in host defence. For water adsorption, glycan variations can greatly affect the osmotic pressures imparted by mucus gels. For example, sialylated and sulfated termini are strongly charged, and their large polar surface areas promote both hydration and electronegative repulsion [11, 13]. In contrast, fucose has a lower charge and an approximately 50% lower polar surface area, which hypothetically promotes mucus aggregation, increases viscoelasticity, and thereby inhibits MCC. For host defence, mucin glycans are known to interact with sugar-binding molecules on a variety of bacteria that colonise or infect the lungs [61–67] and gastrointestinal tract [68–74], on fungi such
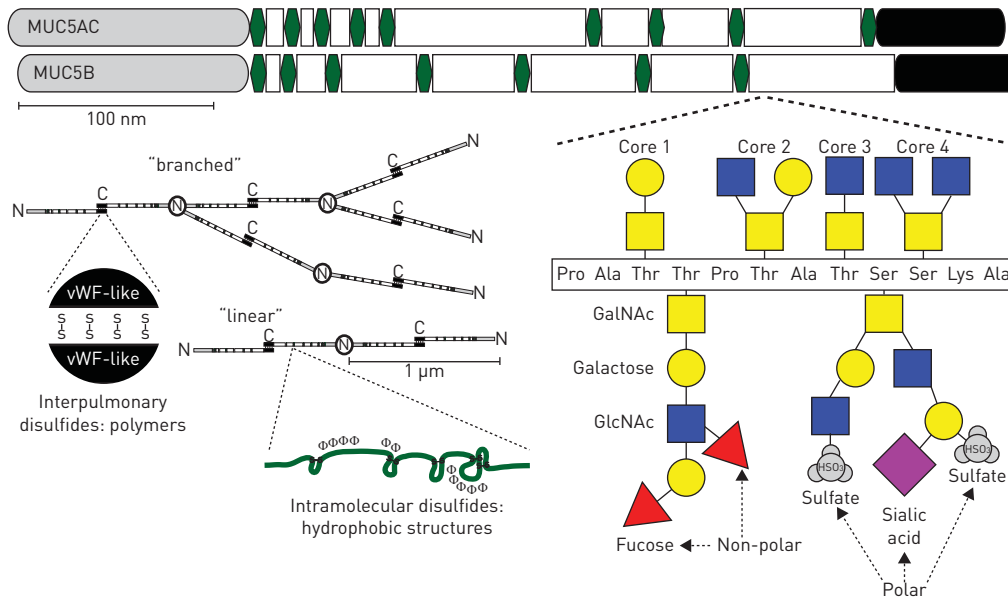
FIGURE 1 Polymeric and macromolecular structures of the major secreted mucins in the airways: MUC5AC and MUC5B. MUC5AC and MUC5B (and their orthologs) have amino (N) and carboxyl (C) termini that are evolutionarily conserved in polymeric mucins and von Willebrand factor (vWF, grey and black regions). The vWF-like domains are involved in covalent intermolecular disulfide assembly of C-terminal linked dimers and N-terminal linked multimers. Multimers can exist as linear or branched structures with sizes in the 1 to >10 MDa range. Between vWF-like domains are additional cysteine-rich regions (CysD domains, green hexagons) that are rich in hydrophobic amino acids (aa) and intramolecular disulfide bonds. CysDs are suggested to mediate the distribution of mucin strands and gel-pore size after secretion in healthy mucus, but can become oxidised and increase in polymer size and stiffness in disease [50]. The majority of the remaining mucin apoprotein backbone is rich in proline, serine and threonine. This "PTS" domain (white) is an imperfect repeat region and is the primary site of O-linked glycosylation. O-linkages on serine and threonine residues form Core 1–4 structures, which are defined the presence of N-acetylgalactosamine (GalNAc, yellow squares) linkages on the hydroxyl groups of serine and threonine followed by single or paired attachments of galactose (yellow circles) and/or N-acetylglucosamine (GlcNAc, blue squares). Lastly, galactose and GlcNAc glycans can be further substituted with fucose, sialic acid and sulfates that impart diverse charges that could affect mucus gel hydration and also form three-dimensional structural confirmations that are critical for interactions with both pathogens and host-cell lectins. Glycan structures shown are examples of possible linkages and do not necessarily represent those found on specific mucins. Polar and non-polar glycans can be found on sugars from each core type, and can be found along the same or different branches.

as *Aspergillus fumigatus* [75], and on respiratory viruses such as respiratory syncytial virus and influenza [76, 77]. Whether these interactions are beneficial to the host or the microbe vary widely. Nonetheless, as the result of host genetics and environmental exposures (such as infectious or allergic states), protection is limited. Impaired defence can be affected by changes in the properties of mucus (*e.g.* through variations in *MUC5AC/Muc5ac versus MUC5B/Muc5b* expression levels or PTS domain glycosylation) that are often coupled with ciliary dysfunction (*e.g.* through loss/absence of ciliated cells or components of motile cilia) [78–91]. Taken together, the roles of mucins in the formation and maintenance of a mucus gel and their abilities to bind microorganisms demonstrate the coordinated function and dysfunction of mucus binding and clearance dynamics in host defence.

In summary, this conventional view of the mucociliary barrier as a defence system regulated by mucus and ciliary functions has been refined by the identification of key factors such as Muc5b and by the dissection of complex biophysical regulation of mucociliary interactions. An immediate challenge is to relate these to specific and required molecular components that regulate their intrinsic biophysical functions. Furthermore, new findings have introduced a novel set of interactions through which mucins regulate defence and inflammation in the lungs *via* resident and recruited pulmonary leukocyte populations. In particular, dendritic cell, eosinophil and macrophage functions in various tissues have been demonstrated to be regulated specifically by mucin terminal glycans. Below we focus on macrophage and eosinophil functions that are regulated by extracellular oligosaccharides, including the airway mucin Muc5b.

Macrophage ontogeny and clearance mechanisms

Particulates and microbes that evade the first line of defence, that is, epithelial mucus, reach the distal lung. From there, they must be cleared rapidly and efficiently by the second line of defence: phagocytes.

AMs are the dominant phagocytic cell in the lungs and during health account for up to 90% of the leukocytes in airspaces [92–95]. They reside in the alveolar lumen and perhaps also in the airways. In addition to clearing inhaled particulates, they are critical for removing dying cells and maintaining alveolar homeostasis. Recent evidence suggests that AMs arise from progenitors that occupy the fetal liver and yolk sac during embryogenesis [96–98]. At birth, these cells populate the airspaces where they quickly mature into resident AMs. Importantly, AMs self-renew throughout life, and in the absence of disease they are not replaced by monocytes from the circulation [99–101]. During inflammation, resident AMs proliferate locally [102]. At the same time, monocytes from the circulation migrate to inflamed regions where they mature into macrophages, termed monocyte-derived AMs (MDAMs) [103]. Hence, the inflammatory AM pool contains cells of both embryonic and postnatal origin. Although both macrophage subsets demonstrate phagocytic capacity, their respective contributions to the clearance of exogenous particulates and pathogens and to the removal of endogenous debris and cells remain unknown. Intriguingly, as inflammation resolves, MDAMs undergo programmed cell death and are removed from the lungs, leaving behind the embryonically derived resident AMs to maintain alveolar homeostasis [103].

During health, resident AMs function as sentinels, constantly surveying the luminal environment for pathogens and inhaled particulates. Under most circumstances, such agents are cleared silently and quickly, without inducing systemic inflammatory responses that could injure alveolar gas exchange structures. Indeed, experimental depletion of AMs results in exaggerated inflammatory responses [104–112], yet at the same time AM absence impairs the ability to control infection [107, 110, 113], demonstrating that restrained responses are more efficacious and beneficial. As discussed below, the alveolar environment plays an essential role in regulating AM endocytic and inflammatory responses, and it also contains a diverse array of molecules that recognise pathogens and facilitates clearance by noninflammatory phagocytic defence.

Phagocytic mechanisms

AMs employ a number of mechanisms to ingest particulates and pathogens, all of which involve endocytosis, a process in which the plasma membrane surrounds a target, invaginates and then pinches off to form a membrane-bound vesicle (reviewed in [114, 115]). Phagocytosis is the primary endocytic process by which AMs clear exogenous materials, and it is driven by cytoskeletal rearrangements that lead to rapid internalisation of pathogens such as bacteria or fungi in a membrane-bound phagosome. The phagosome becomes acidified after sequential fusion with endosomes and lysosomes, which contain hydrolytic enzymes and reactive oxygen species that digest and destroy the target. An initial interface that AMs have with particles and pathogens occurs through a phagocytic synapse formed by a diverse array of plasma membrane proteins that recognise targets through specific moieties on them, including microbial and host cell glycoconjugates. These AM receptors initiate and/or modulate phagocytosis.

Phagocytic receptors

AMs are equipped with a vast repertoire of phagocytic receptors. Importantly, during microbial contact many different receptor families are often simultaneously activated. Some receptors directly recognise specific molecules on phagocytic targets (e.g. phosphatidylserine or inflammasome molecules), whereas others bind to targets coated with opsonins (e.g. immunoglobulins, complement components and surfactant materials). In addition, whereas some (e.g. Fc receptors) lead directly to pathogen engulfment, others (e.g. Toll-like receptors (TLRs)) promote phagocytosis indirectly by upregulating the expression of phagocytic receptors and their downstream signalling molecules [116–118]. Here, we discuss main classes of receptors on AMs in the context of opsonins and signals present in airway mucus [119–125].

Immunoglobulin signalling is an important adaptive immune process that mediates AM phagocytosis. AMs express high levels of Fcγ-receptors I (CD64), II (CD32) and III (CD16) that recognise the Fc region of IgG. Biologically relevant concentrations of IgG can be found in the alveolar lining fluid of healthy humans [126]. To trigger phagocytosis, Fcγ-receptors bind multiple IgG molecules within an immune complex. FcγRI is a high affinity receptor that in addition to respiratory burst and microbial killing also leads to phagocytosis. In comparison, FcγRII and FcγRIII could also promote phagocytosis but have low binding affinity. Respiratory epithelial cells secrete IgA by transcytosis, and IgA can easily be detected in the lumens of both the proximal airways and alveoli [126, 127]. AMs express low levels of both FcαRI (CD89) and Fcα/μR that bind IgA and drive phagocytosis [128]. Adaptive immune immunoglobulin functions are linked to glycan structures through the recognition of carbohydrate antigens, N- and O-glycosylation of their Fc domains, and physical association with secreted mucins that have specific immunoglobulin-binding domains [129–132].

The complement system aids in innate host defence by opsonising immune complexes and pathogens, enhancing their killing and removal. Alveolar lavage fluid of healthy humans contains components of the

classical (C1q, C2, C3, C4) and alternative (C3, Factor B) pathways [133–135]. The classical pathway is primarily activated by the interaction of C1q with antigen–antibody complexes, but it can also be activated by direct binding of C1q to bacterial, fungal and virus membrane components [136, 137]. Opsonisation of targets by either means can stimulate phagocytosis. AMs express three complement receptors (CRs), CR1, CR3 and CR4. CR1 is incapable of internalising opsonised particles on its own, but can enhance Fc-mediated phagocytosis. CR3 and CR4 are heterodimers that share a common β_2 integrin chain (CD18) paired with specific α chains. CR4 contains the α_X subunit (CD11c) and binds to particles opsonised with C3b and iC3b fragments. CR3 contains an α_M chain (also known as CD11b) with a carbohydrate-binding lectin site. Accordingly, in addition to binding particles opsonised with C3b and iC3b fragments, CR3 binds microbial cell wall glycan-containing components including lipopolysaccharide, mannan, β-glucan and others [138, 139]. While CR3 appears to be capable of internalising opsonised bacteria independently [140, 141], it also functions cooperatively with other receptors, including CR1, CD14, FcγR and FcαRI [138, 142–144], to enhance particle clearance. Not surprisingly, mice deficient in CR3 have impaired host defence to Gram-negative bacteria, Gram-positive bacteria and yeast [145, 146]. Importantly, studies from rodents demonstrate that cell surface expression of CRs varies markedly on resident AMs *versus* recruited MDAMs [103]: resident AMs express high levels of CD11c/CR4 but not CD11b/CR3, whereas recruited MDAMs have high levels of CD11b/CR3 but low levels of CD11c/CR4. This raises the intriguing hypothesis that AM subpopulations have complementary functions to control infectious and inflammatory host defence. Like immunoglobulins, complement components are found in airway mucus, and their levels are upregulated in inflammation [147, 148]. Furthermore, complement components also increase the expression of Muc5ac in airway epithelial cells [149].

Other classes of carbohydrate lectins, the C-type lectins, are calcium-dependent carbohydrate-binding proteins that contain a conserved glycan recognition domain and are involved in pathogen recognition and phagocytosis [150]. In the context of lung host defence, two groups of C-type lectins are well recognised: the pulmonary collectins (surfactant protein (SP)-A and SP-D) and the pathogen-binding receptors (namely the mannose receptor (CD206) and Dectin-1). SP-A and SP-D comprise highly oligomerised monomers that are formed by N-terminal collagen-like domains linked to a C-terminal carbohydrate recognition domain by a central hinge region. Through their carbohydrate recognition domains, SP-A and SP-D recognise sugar residues on microbial pathogens. Consequently, they opsonise Gram-negative and Gram-positive bacteria, mycobacteria, fungi, and viruses such as influenza A and respiratory syncytial virus. A number of candidate receptors for collectin-opsonised particles exist on AMs, including C1qRp, SP-R210, CD14 and the calreticulin–CD91 complex (reviewed extensively in [151]). In addition to enhancing phagocytosis through their opsonising effects, collectins could also promote phagocytosis indirectly. For example, SP-A enhances expression of scavenger receptor A and could augment Fc-receptor- and CR-mediated phagocytosis [152–154]. In addition, both SP-A and SP-D appear to increase cell surface localisation and hence the phagocytic function of the mannose receptor [155–157]. The mannose receptor (CD206) is highly expressed on AMs, and contains an extracellular domain that recognises mannose, N-acetylglucosamine and fucose glycans. Accordingly, CD206 promotes phagocytosis of pulmonary pathogens with diverse extracellular carbohydrate signatures, including *Streptococcus pneumoniae, Klebsiella pneumoniae, Mycobacterium tuberculosis, Pneumocystis jirovecii* and fungi such as *Candida* and *Aspergillus* [158]. The precise mechanisms by which CD206 participates in phagocytosis are unclear, and it is likely that interactions with co-receptors are required [159]. Dectin-1 was originally identified as a dendritic cell-specific receptor, but it is also expressed on AMs [160]. Dectin-1 recognises β-glucans found in fungal cell walls [161, 162] and also particles opsonised with pentraxin-3, a protein rapidly synthesised and secreted by mononuclear phagocytes in response to pro-inflammatory signals [163]. Together, these classes of receptors highlight a group of surface molecules that interact with exogenous and endogenous constituents of airway surface liquid and mucus to mediate AM phagocytic defence.

In immunocompetent individuals, defensive components such as IgG increase in the lungs during infection, promoting pathogen clearance through the recognition of numerous antigen types, including carbohydrate epitopes. Indeed, bacterial targets such as surface polysaccharides are exploited for use in developing effective pneumococcal vaccines [164]. Conversely, recurrent sinopulmonary infections and impaired pathogen clearance are common in patients with immunoglobulin deficiencies [165–171]. In addition, in common chronic airway diseases, including asthma, COPD and CF, impaired clearance of microbial pathogens by AMs has been extensively documented [172–175]. AM dysfunction correlates with disease severity and exacerbation frequency [176–178]. While aetiologies vary among diseases, common features include altered expression of phagocytic receptors, reduced lysosomal killing and enhanced production of mediators that can worsen inflammation by inducing collateral damage to surrounding tissues. These defects in AMs are either absent or reduced in mononuclear phagocytes isolated from other sites (*e.g.* blood). Therefore, perturbations in the local environment appear to play a dominant role in altering AM function in these diseases.

Emerging links between airway mucins and alveolar macrophage function

Based on the distinct anatomical localisation and the highly dedicated cellular mechanisms involved in the specification of mucin-producing goblet cells in the airways and phagocytic macrophages in the alveoli, there is an outward appearance of discrete compartmentalisation of their functions. However, the limiting of the localisation of resident AMs to the alveolar space is not entirely warranted, because intraluminal macrophages in conducting airways account for 2–8% of the total resident macrophage population in rat lungs [179–185]. Even within the alveolar compartment, recent evidence demonstrates that a subpopulation of AMs, termed sessile AMs, can communicate across great distances *via* a calcium-dependent signalling AM–alveolar epithelial circuit that ultimately suppresses immune function [186]. Recent studies show that there are indeed functional links between airway mucus and macrophage function, and that these links are crucial for host defence. At one level, secreted factors such as immunoglobulins and complement components are abundant in secreted mucus, suggesting that mucus is an important carrier of these defensive molecules. In addition, there are also direct links between secreted mucins and resident innate immune cells through their coordinated activities when resolving inflammation, and physical interactions between glycans on mucins and carbohydrate-binding lectin receptors on leukocytes such as the sialic acid-binding immunoglobulin-like lectins (Siglec's). We propose that mucin–leukocyte interactions regulate homeostatic, inflammatory and resolving immune functions through signalling and physical clearance mechanisms (figure 2).

In the mouse, the intestinal mucin Muc2 interacts with glycan-selective immunoregulatory receptors on dendritic cells that mediate the development of inflammatory and regulatory lymphocyte subsets. In this setting, Muc2 glycans bind to two lectins (Dectin-1 and Galectin-3) that function cooperatively with the inhibitory IgG receptor FcγR3 to suppress inflammatory signals and promote tolerance [187]. In a similar vein, goblet cells have also been shown to be an important mechanism for the delivery of antigens to resident monocyte-derived dendritic cells in the small intestine [188]. The result of these activities is the development of tolerance to foreign antigens introduced by ingested food particles.

In the lungs, inhibitory regulation of leukocyte functions appears to be mediated by acute control of leukocyte activation states. In mice, Muc5b binds through its α2,3-linked sialoside glycans to Siglec-F, an inhibitory SH2 domain-containing-phosphatase signalling immunoreceptor on eosinophils and AMs (figure 3) [189]. On eosinophils, Siglec-F mediates apoptosis [190–193], thereby functioning as a significant mechanism for resolving allergic inflammation. Indeed, mice lacking Siglec-F or one particular enzyme needed for this Muc5b sialylation step, ST3Gal-III, fail to make airway ligands for Siglec-F, and display exaggerated and selective lung eosinophilia in a type 2 allergic inflammation lung model [194–198]. In this context, Muc5b presumably contributes to the physical removal of cells by MCC while simultaneously preventing continued activation and mediator release into airspaces during elimination from the mouse lung. In humans, the Siglec-F paralog Siglec-8 also reduces eosinophil survival *via* sialylated and sulfated
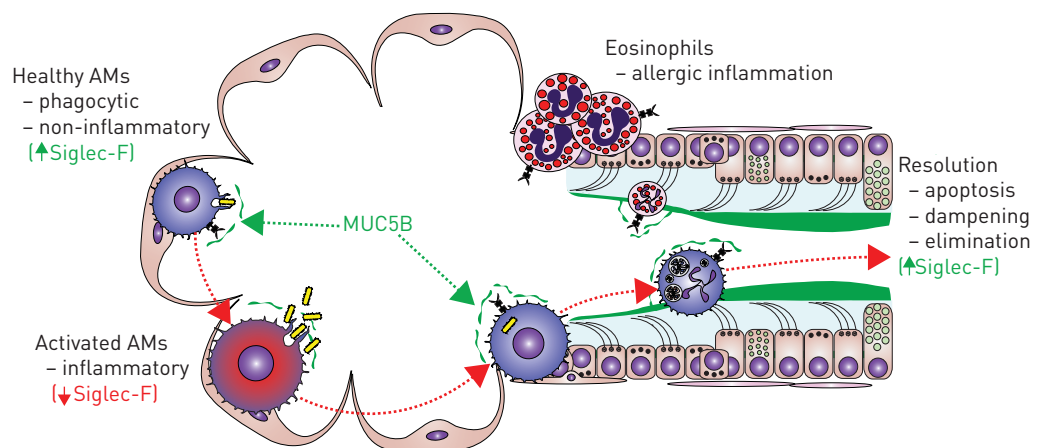

FIGURE 2 Mucin–leukocyte interactions during homeostasis and inflammation. In healthy lungs, resident resting alveolar macrophages (AMs) are defensive and noninflammatory. MUC5B from bronchioles mixes with alveolar fluids, providing a route for MUC5B to contact alveolar AMs. Homeostatic or low-dose stimuli elicit defensive functions such as phagocytosis. During inflammation, resident AMs can become activated, and this is associated with a decrease in their Siglec-F surface expression. In addition, leukocytes, such as monocyte-derived macrophages (which lack Siglec-F) or eosinophils (which express Siglec-F) are recruited and persist for brief periods of time. These transient populations are eliminated as inflammation resolves. In mice, resolution involves Siglec-F-mediated reductions in leukocyte activation and survival. Damaged and apoptotic cells are subsequently eliminated by MUC5B-mediated mucociliary clearance.
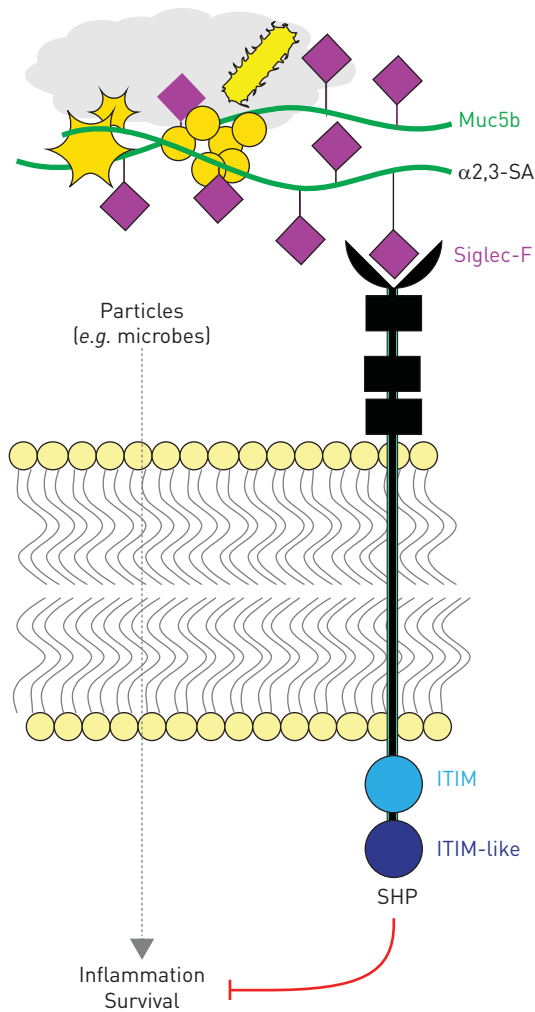
FIGURE 3 Putative Muc5b:Siglec-F signal transduction mechanism. Muc5b, via its display of multivalent α2,3-sialic acid (SA) linkages on galactose residues, binds to the N-terminal lectin domain of Siglec-F, thereby driving immunoreceptor tyrosine-based inhibitory motif (ITIM) and ITIM-like domain activation. ITIM signals putatively activate Src homology region 2 domain-containing phosphatase (SHP) enzymes that suppress kinase-activated inflammatory signals and can also promote apoptosis.

ligands, but the specificity observed between Muc5b and Siglec-F in mice is not as well conserved between MUC5B and Siglec-8 in humans [199–201]. Rather, Siglec-9 is an isoform that is bound by MUC5B sialosides, and it is expressed on neutrophils, natural killer cells, dendritic cells and monocytes/macrophages [199]. Indeed, resident AMs in healthy mouse lungs also express Siglec-F, but its role beyond that of a cell surface marker is not yet clear. Given the associations of mucus and macrophage dysfunction in numerous lung pathologies, determining the nature of their interactions will be of tremendous interest as the field advances. With the emergence of mucins as important mediators of defence, and the recognition of the crucial significance of the glycobiology of innate and adaptive immunity, efforts to interrogate these will involve both challenging and exciting experimental approaches.

Conclusion

Innate defences in the lungs are essential for maintaining efficient gas exchange. As first and second lines of host defence, mucins and macrophages play critical roles that are integrated by their physical and physiological interactions. The emergence of these links presents a convergence of new challenges that connect epithelial and innate immune programmes.

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