Immune responses to bacterial lung infections and their implications for vaccination

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1 Figure

1 Table

Abstract

The pulmonary immune system plays a vital role in protecting the delicate structures of gaseous exchange against invasion from bacterial pathogens. With antimicrobial resistance becoming an increasing concern, finding novel strategies to develop vaccines against bacterial lung diseases remains a top priority. In order to do so, a continued expansion of our understanding of the pulmonary immune response is warranted. Whilst some aspects are well characterised, emerging paradigms such as the importance of innate cells and inducible immune structures in mediating protection provide avenues of potential to rethink our approach to vaccine development. In this review, we aim to provide a broad overview of both the innate and adaptive immune mechanisms in place to protect the pulmonary tissue from invading bacterial organisms. We use specific examples from several infection models and human studies to depict the varying functions of the pulmonary immune system that may be manipulated in future vaccine development. Particular emphasis has been placed on emerging themes that are less reviewed and underappreciated in vaccine development studies.

Introduction

With each breath, the respiratory system is continuously challenged by harmful pathogens and foreign materials. Therefore, the pulmonary immune system acts in a controlled manner to mount sufficient protection whilst avoiding significant damage to the delicate structures vital for gaseous exchange (1). As the lower respiratory tract is an interface with the atmosphere and therefore a portal of entry for several bacterial pathogens, the respiratory system has several immune defences to maintain its homeostatic environment. Thus, we review these immune defences utilised by the respiratory system (Fig. 1) and discuss emerging concepts of pulmonary immunology (Table 1) that may be targeted in future vaccine development.

Pneumonia refers to severe forms of acute lower respiratory tract infections and remains one of the leading causes of death worldwide (2). Bacterial pneumonia is classified as either community- or hospital-acquired and is caused by typical (*Streptococcus pneumoniae, Haemophilus influenzae* and *Klebsiella pneumoniae*) or atypical (*Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and *Pseudomonas aeruginosa*) organisms (2). Of significance is *Mycobacterium tuberculosis,* the causative agent of tuberculosis (TB), and the most prevalent bacterial lung infection worldwide (3). Although routine vaccination has resulted in major reductions in the incidence of some bacterial infections, the increasing prevalence of chronic lung diseases [chronic obstructive pulmonary disease (COPD), bronchiectasis etc.] and immunocompromised states is linked to increases in opportunistic bacterial infections, including *Pseudomonas spp.* and non-tuberculous mycobacteria (2). This, coupled with the continual rise of antibiotic resistance (4), supports development of new vaccines as an attractive solution for prevention of bacterial lung infections. However, the unique immunological landscape of the lungs poses several challenges to vaccine-acquired pulmonary immunity. In this review, emphasis is placed on select bacterial species that are either prevalent cause of lung infection worldwide or of increasing public health concern. Immune responses will be discussed in general, or in relation to specific pathogens across animal and human studies.

Physical and chemical barriers

The respiratory system is divided into two anatomical compartments: upper (nasal cavity, nasopharynx and trachea) and lower (bronchi, bronchioles and alveoli) airways (5). The trachea

bifurcates into right and left main bronchi, which subsequently branch to terminate into alveoli where gaseous exchange occurs. The functions of the upper airway of thermoregulation, humidification and filtration protect the lower delicate tissues from the harsh external environment (5). To assist this process, the upper airway mucosa is composed of ciliated pseudostratified epithelium and mucus-producing goblet cells to create a well-formed physical barrier between the external environment and underlying tissues (6). As thousands of bacteria and other antigens are inspired, mucus traps these foreign materials, while the continual sweeping action of the cilia directs potentially harmful material away from the lower respiration tract. This 'mucociliary escalator' (Fig. 1) is the first line of defence the respiratory system uses to maintain a germ-free environment (6).

Fliegauf *et al*.(7) demonstrated that *S. pneumoniae* disrupts the mechanics required for effective ciliary beating, thus distorting physical clearance by this escalator action in mice. Furthermore, inherited or acquired diseases such as primary ciliary dyskinesia, cystic fibrosis, asthma, COPD and bronchiectasis, which disrupt the clearance of mucus, predispose individuals to mycobacterial and *P. aeruginosa* infection (8), further highlighting the importance of this physical defence mechanism (6).

The structural integrity of the respiratory epithelium also plays a key role in infection prevention. Through tight junctions formed by adherens (β-catenin and E-cadherin), occludins and claudins, the epithelium forms a near-impenetrable surface to many bacteria (9). The mucin glycoproteins MUC5AC and MUC5B, found in secreted mucus have an additional anti-bacterial and immunomodulatory role (10,11). Respiratory epithelial cells also secrete a range of antimicrobial and host defence peptides including defensins, surfactant proteins and enzymes (12). LL-37 cathelicidin produced by respiratory epithelial cells and innate immune cells also has broad-spectrum antibacterial activity against gram-positive (*Staphylococcus aureus,* Group A *Streptococcus, Enterococcus faecalis*) and gram-negative bacteria (*K. pneumoniae, P. aeruginosa, Burkholderia pseudomallei*) (12,13).

Innate cellular immunity

Macrophages and dendritic cells

87 Bacterial invasion is detected by pattern recognition receptors (PRRs) on innate immune cells, which
88 recognise conserved non-self molecules, such as endotoxins, flagella, lipopolysaccharide,
89 peptidoglycans and nucleic acids, collectively called pathogen-associated molecular patterns
90 (PAMPs) (14).
91 Macrophages express a vast array of PRRs and are typically the first cells of the immune system to
92 respond to bacterial invasion (Fig. 1). Amongst alveolar and interstitial macrophages (15), alveolar
93 macrophages ($CD11c^{+}CD11b^{-}MHCII^{lo}CD206^{+}$) serve as sentinel phagocytes of the airspaces and
94 are highly specialised to the lung environment. To minimise damage to alveolar structures, these cells
95 are tightly regulated under steady-state conditions to limit dendritic cell (DC) access to antigens and
96 directly suppress antigen presentation by lung DCs to avoid unnecessary adaptive responses (16).
97 Interstitial macrophages represent a conserved group of phagocytes ($CD11b^{+}MHCII^{+}$ or
98 $CD11b^{+}MHCII^{low}$) that are found in the alveolar interstitium and peribronchiolar spaces. Traditionally,
99 macrophages were categorized into two phenotypes: classically activated M1 macrophages (mediate
100 defensive functions) or alternatively activated M2 macrophages (responsible for tissue repair) (17).
101 Recent advances in the field demonstrate that macrophages switch between phenotypes, depending
102 on the tissue milieu, with intermediate phenotypes observed.
103 The large surface area of macrophages allows them to rapidly phagocytose inhaled particles
104 (including bacteria) and subsequently degrade them via reactive oxygen species (ROS) and nitric
105 oxide (NO) in secondary lysosomes (18). Degradation of bacteria via these processes further
106 stimulates cytosolic and membrane-associated PRRs to enhance inflammatory signals. Interestingly,
107 some bacteria such as *S. pneumoniae* have developed mechanisms to resist oxidative stressors and
108 use hydrogen peroxide production within macrophages to limit replication of other competitive
109 bacterial species (19). Experimental depletion of alveolar macrophages in mice results in significant
110 growth of *S. pneumoniae* and *K. pneumoniae* following inoculation and requires the recruitment of
111 polymorphic nuclear cells to control bacterial replication (20,21). In instances where macrophages
112 are overwhelmed by the amount of bacteria phagocytosed, apoptotic responses occur to limit survival
113 of engulfed/intracellular bacteria (18). Finally, secretion of IL-8 and CXCL5 by alveolar macrophages,

along with chemokines released by epithelial and endothelial cells drives neutrophil chemotaxis into the lung to assist with phagocytosis.

Whilst macrophages dominate initial phagocytosis, pulmonary DCs are also able to recognise PAMPs and clear bacteria, albeit to a lesser extent, once macrophages are overwhelmed (16). The main role of DCs, however, is antigen presentation that serves as the key bridge between innate and adaptive immunity. The lung DC population consists of two groups: conventional DCs (cDCs, which have a further 3 subgroups: $CD103^+$ cDCs, $CD11b^+$ cDCs and plasmacytoid DCs) and monocyte-derived DCs (mDCs) (16). Once they have internalised bacteria, pulmonary DCs migrate to draining mediastinal lymph nodes to interact with the adaptive immune system (Fig. 1) (15).

Neutrophils

Although not routinely found within the airways under steady-state conditions, neutrophils police the pulmonary vasculature. They are crucial in controlling bacterial infections, as several experimental depletion studies demonstrate a significant reduction in bacterial clearance during lung infection (22). Furthermore, neutropenia is associated with opportunistic bacterial lung infection. Conversely, neutrophils are also implicated in pneumonia progression and acute lung injury (22) because of their cellular processes and their chemical arsenal that is required for pathogen destruction.

Once recruited to sites of lung infection, neutrophils rapidly phagocytose bacteria to limit replication, and use cytotoxic granules to kill them. Neutrophil granules are categorised into four groups based on protein content (23). Secretory granules contain key membrane proteins to facilitate extravasation from blood vessels into sites of infection. Primary, secondary and tertiary granules contain antimicrobial chemicals that are either released into surrounding tissues or phagolysosomes that contain endocytosed bacteria. Within neutrophil phagolysosomes, phagocytosed bacteria are also neutralised by ROS (O_2^- and H_2O_2) through oxidation of bacterial proteins, lipids and nucleic acids. Neutrophil-associated ROS, however, can cause significant host tissue damage (24). Therefore, tight control of neutrophil activity is needed within the lung to avoid major tissue damage.

Another neutrophil-associated mechanism of bacterial control is formation of neutrophil extracellular traps (NETs), whereby neutrophils release their DNA into large woven fibres to entrap and

subsequently kill bacteria (Fig. 1) (25). NETs contain histones, elastases, MMP-9 and serine proteases, which degrade microbe virulence factors and assist with their elimination. The significance of NETs is demonstrated by primary and acquired NET deficient states that increase host susceptibility to bacterial infection (26-28). Interestingly, EndA, a nuclease produced by *S. pneumoniae,* is able to degrade NET DNA scaffolding, which allows bacterial escape (29).

Resolution of inflammation, following neutrophil apoptosis, is necessary to prevent further damage to lung tissue following inappropriate release of neutrophil granule proteins and ROS (22). Subsequently, bystander scavenger macrophages eliminate neutrophil debris via efferocytosis, allowing local tissue repair to begin in a timely manner.

Donor-unrestricted T cells

The finding that a number of T cells are activated through non-MHC antigen-presentation mechanisms led to the characterisation of a diverse range of innate-like T cells (30). MHC-related protein 1 (MR1), a highly conserved molecule, presents microbial by-products such as 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil from vitamin B (riboflavin) pathways to mucosal associated invariant T (MAIT) cells (31). Another MHC I-related molecule, cluster of differentiation 1 (CD1), presents mammalian and microbial lipids; thus T cells that recognise lipid antigens through this pathway are referred to as CD1-restricted T cells (subtypes include group 1 CD1a, CD1b, CD1c; and group 2 CD1d) (32). γδ T cells (with T cell receptors composed of γ and δ chains) use butyrophilins to recognise phosphoantigens (33).

Because of the non-polymorphic nature of these antigen-presenting molecules MR1, CD1 and butyrophilins, cells that utilise them are not constricted to the genome of their donor. Furthermore, the T-cell receptor (TCR) of MAIT and CD1-resticted T cells is partly invariant and thus found in unrelated individuals. Therefore, this group of innate-like T cells has been termed donor-unrestricted T cells (DURTs) (30). Although only accounting for around 15% of circulating T cells in humans, DURTs are abundant in peripheral barrier tissues including the lungs (32); hence, they fulfil a unique role in mucosal immunity at the interface of innate and adaptive immune systems. Unlike their conventional

169 T cell counterparts, DURTs are capable of mounting rapid effector cytokine and chemokine release
170 and cytolysis following thymic egress (33).
171 Until recently, research into DURTs was hindered by our inability to identify these cells, as well as the
172 differences between animal and human DURTs. It is beyond the scope of this review to discuss the
173 novel biology of these DURTs. Nonetheless, their importance in protection against bacterial lung
174 infection is an emerging topic of interest for vaccine development (32).
175
176 *MAIT cells*
177 MAIT cells take residence in the lung, where they patrol tissue during steady-state conditions (Fig. 1)
178 (34). In response to TCR signals and a range of activating cytokines, MAIT cells secrete Th1 and
179 Th17 cytokines to effectively kill bacterially infected cells (31). Specifically, lung MAIT cells of mice
180 display a phenotype of IL-17^{+} cells and generate strong IL-17A release (35), although Th1 phenotypes
181 have also been observed (36). MAIT cells also express a CD44^{+}CD62L^{-} phenotype that mimics
182 effector memory cells (35) and demonstrate a role in anti-mycobacterial immunity in both mice and
183 humans. In an IL-12-dependent fashion, mouse MAIT cells secret IFN-γ, to inhibit *M. bovis* BCG
184 replication in macrophages. It is thought that MAIT cells play a role in early control of *M. bovis* BCG
185 infection, as MAIT knockout (*Mr1*$^{-/-}$) mice have decreased capacity to control bacterial replication
186 when compared with wild-type mice. Moreover, enhanced bacterial control was not evident in later
187 stages of infection (37).
188 Interestingly, the numbers of circulating MAIT cells are significantly reduced in patients with active TB
189 in comparison with healthy controls. Analysis of lung tissue and pleural fluid of TB patients, however,
190 revealed an abundance of MAIT cells, suggesting that they may be recruited from the circulation into
191 the lung parenchyma during infection (38). Importantly, MAIT cells from pleural fluid of TB-infected
192 patients produce large amounts of IFN-γ and TNF-α (39) and have been observed to mediate
193 cytotoxicity against *M. tuberculosis* and other bacteria in infected lung epithelial cells and DCs (38).
194 *In vitro*, MAIT cells also demonstrate bacterial control in *Francisella tularensis* live vaccine strain
195 (FTLVS)-infected monocytes in an IFN-γ, TNF-α and NO dependent manner. Furthermore, *Mr1*$^{-/-}$

196 mice suffer higher bacterial burden compared with C57BL/6 wild-type mice after FTLVS pulmonary
197 infection (40). Mice infected with *F. tularensis* generate robust populations of MAIT cells of Th1 and
198 Th17 phenotypes that remain 100 days post-infection (41). *Mr1*$^{-/-}$ mice also show increased
199 susceptibility to *K. pneumoniae* infection (42). However, MAIT cells do not play a significant role in *S.*
200 *pneumoniae* infection despite transcriptomic analysis that indicates that several strains of *S.*
201 *pneumoniae* express riboflavin-synthesising enzymes (43). Finally, through systemic priming with
202 synthetic MAIT antigen (5-OP-RU and CpG adjuvant), Zhao *et al.*(36) demonstrated that MAIT cells
203 in the lung can be skewed towards a Th1 phenotype to enhance bacterial clearance of FTLVS and
204 *Legionella longbeachae*, perhaps a strategy that can be employed in vaccines.
205
206 *CD1-restricted T cells*
207 Our understanding of CD1-restricted T cells has been largely confined to group 2 CD1d natural killer
208 T cells (NKT cells), as group 1 CD1a, CD1b and CD1c are not found in mice (44). CD1d-restricted T
209 cells represent a heterogeneous group of three sub-groups. Type I (invariant) NKTs largely recognise
210 and respond to the glycosphingolipid alpha-galactosylceramide and other microbial lipids and account
211 for ~3% of T cells in murine tissues and 0.1% of T cells in human (33). Type II (variant) NKTs react
212 to lipids, but are not reactive to alpha-galactosylceramide. Other subtypes have been recently
213 identified and are classed as ‘other’, a third sub-group (45).
214 CD1d-restricted T cells have effector functions including cytokine secretion and cytotoxicity. Like
215 many bacteria, the cell wall of *S. pneumoniae* contains glycolipids, providing key antigenic targets for
216 NKT cells (46). As such, NKT cells, particularly type I, play a vital function in protection against *S.*
217 *pneumoniae* (Fig. 1), by recruiting neutrophils and secreting IFN-γ (47). They have also demonstrated
218 capabilities of assisting B cells to generate anti-pneumococcal antibodies (48). During *C. pneumoniae*
219 infection, type I NKT cells activate DCs and induce IFN-γ production by conventional T cells, to
220 enhance protection (49). However, the protective capabilities of NKT cells vary greatly. For example,
221 several studies report early activation of type I NKT cells during infection with *M. bovis* BCG and *M.*
222 *tuberculosis* (50), where they have a role in mycobacterial control (51). On the other hand,

223 experimental models of pulmonary tularaemia suggest that NKT cells exacerbate disease progression
224 (52).
225 Lastly, research into group 2 CD1-restricted T cells focuses largely on *M. tuberculosis* where recent
226 developments in CD1a–c tetramer technology has allowed characterisation of a number of
227 mycobacterial antigens (32). The protective capacities of these subsets warrants further investigation.
228
229 *γδ T cells*
230 γδ T cells rapidly detect conserved non-peptide antigens and mount early effector responses. They
231 also recognise stress-induced MIC-A and MIC-B ligands, isoprenoid pathway-derived peptide
232 antigens and mevalonate pathway signals of infected cells (53). γδ T cells are able to migrate to
233 barrier tissues and take residence (53), thus are ideally suited to perform immunosurveillance (Fig.
234 1). During *S. pneumoniae* infection, γδ T cell numbers in the lung are significantly bolstered and serve
235 as an important source of TNF-α and IL-17A (54). Furthermore, adoptive transfer studies demonstrate
236 that *S. pneumoniae*-cognisant γδ T cell have superior lung-homing capabilities compared with αβ T
237 cells (55). Similarly, in *K. pneumoniae* infection γδ T cells produce large amounts of IL-17A, although
238 this is age-influenced in mice (56). γδ T cells play a central role in innate responses to pulmonary TB,
239 as well as continued modulation of later immune responses through IL-17A-associated maturation of
240 granulomas (57).
241
242 **Adaptive immunity**
243 *T cells*
244 The crucial role of conventional T cells in pulmonary defence against bacterial lung infection is well
245 established. Much of our understanding centres on the Th1, Th2, Th17 and Treg paradigm of T cell
246 biology, extensively reviewed elsewhere (Fig. 1) (58). The adaptive T cell response to lung infection
247 is largely orchestrated by primed T effector memory cells (T_{EM} cells) that are recruited from circulation.
248 However, the discovery of non-circulating tissue-resident memory T cells (T_{RM} cells) that take
249 residence in peripheral tissues to carry out immunosurveillance and rapid effector responses has
250 garnered much attention (Fig. 1) (59). T_{RM} cells display a number of phenotypes including those of

CD4 and CD8 subsets and confer better protection against several bacterial lung infections compared with T_{EM} cells (60,61).

Mice infected with *S. pneumoniae* produce a strong $CD4^+$ T_{RM} cell response in pneumonia-affected lobes, where immunity remains localised (62). A similar phenomenon occurred in mice infected with *Bordetella pertussis* where investigators used FTY720 (an inhibitor of tissue infiltration by T cells) and adoptive transfer to demonstrate that bacterial control could be achieved independently of T_{EM} cells (63). The protective capacities of T_{RM} cells in the context of TB have also been described (60). Analysis of human tissues revealed that T_{RM} cells accumulate in high frequencies at sites of TB infection and have a strong capacity to limit *M. tuberculosis* replication within macrophages (64).

In many tissues such as skin, the female reproductive tract and the gastrointestinal tract, T_{RM} cells are known to persist for many years (59). In the lungs, however, T_{RM} cells do not persist (64) and this is a major limitation for their protective role against bacterial lung infections. One hypothesis is that the lung tissue may foster an immunosuppressive environment through the local cytokine milieu, expression of co-inhibitory molecules and epigenetic regulation, to minimise the risk of unnecessary and noxious immune activation. Finding strategies to generate and retain T_{RM} cells in the lungs safely should be a goal of new vaccines targeting lung bacterial pathogens. For example, although there is emerging evidence that viral lung infections lead to the establishment of repair-associated memory depots (RAMD) in the lung (65), currently there is no evidence that these structures also form after bacterial lung infections.

B cells

The role of B cells and protective antibodies has been well described in mucosal tissues including the lungs (66). During early bacterial infection, innate-like B1a B cells migrate into the lung parenchyma from resting sites in pleural spaces and mount a non-specific, polyreactive immunoglobulin M (IgM) response in a GM-CSF-dependent autocrine fashion (Fig. 1). In a murine model, Weber *et al*.(67) demonstrated that early IgM production protects against bacterial pneumonia. Secretory immunoglobulin A (sIgA) antibodies have a much broader binding capacity than immunoglobulin G

(IgG) counterparts and neutralise bacteria at luminal surfaces of airways in a process called immune-exclusion (68).

The utility of IgA secretion relies on close interaction of mucosal lymphoid cells (such as plasma cells) for dynamic, continuous production of specific/non-specific IgA, and on epithelial cells that transport IgA into the airway lumen via the polymeric immunoglobulin receptor (68). However, several species of bacteria have developed evasion tactics against sIgA. For example, virulent strains of *H. influenzae* and *S. pneumoniae* produce proteases that cleave IgA (69,70)**.**

Recently, resident memory B cells (B_{RM} cells) have been identified as a common component of the adaptive pulmonary immune system in both humans and mice. These non-circulatory B cells express a unique CD80, CD69, CD73 and PD-L2 surface-marker phenotype (71) and, much like their T_{RM} cell counterparts, remain poised to mount rapid effector responses to secondary exposure to antigen (Fig. 1). Lung tissue recovered from pneumococcus-infected mice contain B_{RM} cells that demonstrate a heterotypic anti-pneumococcal protective function and take residence in disorganised peri-vascular and bronchiolar clusters situated in close proximity to $CD4^+$ T cells (71).

Inducible structures and tissues

In response to bacterial infection, mucosal tissues generate highly organised inducible lymphoid structures (72). Within the lungs, two well-defined tertiary lymphoid structures, namely inducible bronchus-associated lymphoid tissue (iBALT) and granulomas, develop during and/or after bacterial infections (73,74). Both of these structures are correlated with enhanced protection against bacterial infections, particularly TB.

The iBALT forms at sites of infection in close proximity to the basement membrane of the bronchiolar epithelium, adjacent to surrounding pulmonary vasculature (Fig. 1). The inflammatory milieu that stimulates the generation of iBALT is initiated by epithelial, endothelial and stromal cells, subsequently reinforced by recruited macrophages and DCs, where CCL19, CCL21, IL-17, IL-22 and IL-23 are signals required for their organisation (72,73). As B and T cells accumulate at the forming iBALT, the nascent structure matures to form a distinct central B cell follicle (germinal centre-like structure) and a surrounding T cell zone, reminiscent of secondary lymphoid organs.

306 The iBALT is thought to maintain a local population of antigen-specific lymphocytes poised to mount
307 rapid effector responses (72,73). In line with this hypothesis, iBALT has been associated with
308 enhanced protection against chronic *M. tuberculosis* infections. Using a non-human primate model,
309 Slight *et al*.(75) demonstrated that enhanced immune control of latent TB was associated with the
310 presence of highly organised iBALT. In support of this observation, aerosol vaccination of macaques
311 with an attenuated *M. tuberculosis* strain (Mtb∆*sigH*) stimulates significant iBALT formation and
312 enhances protection against lethal challenge with TB (76). Furthermore, a protective role for iBALT
313 has been demonstrated in *P. aeruginosa and F. tularensis* infections (77,78). Although stimulation of
314 iBALT formation is an attractive goal for novel vaccine development, iBALT has also been linked to
315 several pathological states such as COPD (73). Thus, delineating the defining characteristics between
316 protective and pathological iBALT is a paramount step forward in the field of pulmonary immunology.
317 Granulomas of the lung have also been widely researched, particularly in the context of TB (79).
318 Traditionally, granulomas have been viewed as a protective response against TB as they 'wall off'
319 bacteria, to initially prevent dissemination of *M. tuberculosis.* The concept of mycobacterial
320 containment within granulomas is supported by the demonstration of the migration of *M. tuberculosis*-
321 specific T cells to the site of mycobacterial replication, engagement with infected macrophages via
322 IFN-γ and TNF-α to stimulate mycobactericidial activity and eventual granuloma formation to contain
323 residual mycobacteria (79). Further support lies in models of knockout mice that demonstrate that a
324 lack of CD4 T cells, TNF-α and/ or IFN-γ results in loss of *M. tuberculosis* containment (80,81).
325 Recent advances, however, have demonstrated a role for granulomas to enhance the survival of
326 mycobacteria within the host. Whilst the mechanisms are still not fully understood, several host-
327 detrimental features of granulomas have been elucidated. For example, $CD11c^+$ DCs have been
328 observed to migrate in and out of granulomas, to potentially act as a shuttle for mycobacteria into
329 surrounding tissue (82). Moreover, as TB disease progresses, exhausted macrophages of
330 granulomas undergo necrosis, spilling mycobacteria into the growth-permissive extracellular
331 environment (83). Enhancement of the protective features of granulomas, while minimising bacteria-
332 beneficial mechanisms, may lead to better control of latent mycobacterial infections of the lung.
333

Clinical perspectives and vaccinations strategies

The World Health Organisation recommends routine immunisation against *B. pertussis*, *H. influenzae* type B and *S. pneumoniae* (84). Since the introduction of these highly effective vaccines, the incidence of bacterial infections caused by these organisms has drastically reduced, although *S. pneumoniae* remains the leading cause of severe pneumonia partly due to the existence of several non-vaccine preventable serotypes (85). Furthermore, species such as *Legionella*, *C. pneumonia* and *M. pneumonia*, for which no licenced vaccines are available, have emerged as prevalent causes of bacterial pneumoniae (86). TB continues to remain as one of the most significant bacterial lung infections to threaten global human health. Although BCG is still routinely administered in areas of high TB prevalence, attempts to develop a more effective vaccine have proven to be difficult (87). There are several challenges in development of new vaccines against TB and emergent pulmonary pathogens.

Previously, much focus has been placed on humoral immunity (88), given the success of several routinely administered licenced vaccines. However, there is a clear need to delineate other pulmonary immune defences and how these may be stimulated through novel vaccine technologies to generate protection against bacteria. Several emerging strategies are being explored. Particularly, the vaccine delivery route is demonstrated to be an influential factor in the type of immune responses generated. In many cases, matching the route of vaccination to the route of pathogen entry induces stronger localised protection and thus mucosal vaccination has become an increasingly popular concept (89). Intravenous and mucosal vaccination has also demonstrated the capacity to enhance ‘trained immunity’ (immune memory of the innate immune system) in animal models of TB (90,91).

Previously, vaccine efficacy studies largely focused on strategies that bolster components of the immune system that have been well researched, such as antibody generation and conventional T cell immunity. Increasing evidence demonstrates that other cells types such as DURTs respond more rapidly than conventional T cells or B cells, and the important role of mucosal lymphoid tissues highlights the need to explore these alternative cell functions.

Finally, utilization of adjuvants/chemokines that stimulate protective responses from these immune cells and structures may be a worthy strategy to pursue (92). For example, “prime and pull” or “prime

and trap" strategies, where protective cells are induced (primed) and a range of strategies are implemented to pull/trap these cells at the barrier tissues where they are needed, may be expanded (93,94). The coronavirus disease 2019 (COVID-19) pandemic and rapid development of mRNA vaccine technologies have also created exciting potential to rethink vaccine development against bacterial infection (95).

Conclusion

The lungs are a significant portal of entry for several bacterial pathogens. Although a myriad of host defences can be mounted, the importance of the immune system to act in a controlled manner poses dilemmas. Delineation of methods to manipulate highly active pulmonary-resident immune cells such as DURTs and T_{RM} cells may present a novel method to enhance immune protection within the lungs. Furthermore, elucidation of specific cellular subsets of the adaptive immune system may allow for the development of strategies that are tailored to circumvent the sophisticated immune-escape tactics employed by various bacterial pathogens. New vaccines should aim to utilize these advances in pulmonary immunology to protect against new and emerging bacterial lung diseases of public health importance.

383 **Table 1: Pulmonary Immune Responses to Specific Bacterial Pathogens**

Pathogen	Innate		Adaptive		
	Macrophage/DC/ Neutrophils/ Chemical defenses	MAIT/CD1-restricted/γδ T cells	Tcell	B cell/humoral	Immune structures/ Clinical implications
S. pneumoniae	**Human:** •IL-17 stimulates macrophage/neutrophil killing of *S. pneumoniae*(96) •*S. pneumoniae* Pneumococcal surface protein C prevents complement deposition by binding to factor H(97) •Microinvasion of the epithelium by *S. pneumoniae* enhances innate responses(98)	**Human:** •13-valent pneumococcal conjugate vaccine induced antibodies enhance antigen presentation to MR1-restricited cells, resulting in more effective responses on stimulation(99) •MAIT cells play a role in protecting against *S. pneumoniae* colonization(100)	**Human:** •*In vitro* mixture of *S. pneumoniae* whole cell antigen with blood from unimmunised adults resulted in IL-17A production(96) •IL-17A responses by memory T cells are elicited by stimulating adenoidal tissue and PBMC with *S. pneumoniae* antigens *in vitro*(101)	**Human:** •Polyreactive monoclonal IgM antibodies are found in the serum of healthy young adults, that are capable of binding to several serotypes of *S. pneumoniae*(102) •Experimental colonization with *S. pneumoniae* increased anti-pneumococcal IgG antibodies in the upper respiratory tract and serum, conferring protection against experimental challenge(103) •Naturally acquired secretory IgA against pneumococcal pilus-1 mediates immune exclusion(104)	**Human:**
	Animal models: •Neutrophils are essential for optimal development of protection mediated by vaccines against *S. pneumoniae*(105) •TLR4 signalling is vital in the pulmonary innate response to *S. pneumoniae*(106) •Alveolar macrophages from TLR7/9/13 Triple KO mice are unable to recruit neutrophils during *S. pneumoniae* infection(107)	**Animal models:** •γδ T cells modulate macrophages and neutrophil activity following *S. pneumoniae* infection in mice(55) •IL-10 secretion by NK cells in the lungs results in exacerbation of *S. pneumoniae* infection in mice(108)	**Animal models:** •IL-17A produced by $CD4^+$ T cells is protective against *S. pneumoniae* in mice(96) •$CD4^+$ T_{RM} generate in lung lobes affected by *S. pneumoniae* pneumonia(62)	**Animal models:** •B cell depletion in mice results in reduced antibody recognition of *S. pneumoniae*, however these mice still maintained some level of immunity(109)	**Animal models:** •Alpha-1 antitrypsin KO mice are more susceptible to *S. pneumoniae* compared to wild type mice(110) •Repeated exposure to *S. pneumoniae* in the presence of IL-33 induces asthma like pathology and iBALT in mice(111)
H. influenzae (H. influenzae B, HiB and nontypable, NTHi)	**Human:** •Lung fibroblasts can internalise NTHi and are able to present antigens to and activate $CD4^+$ T cells via HLA-DR(112) •NTHi stimulates the production of ROS by fibroblasts, epithelial cells, macrophages and neutrophils. Macrophage extracellular trap-like structures are also observed(113)	**Human:** •Steroid induced deficiency of MAIT cells in the airways of COPD patients may increase susceptibility to NTHi(114) •IL-12 and IL-17 modulate MAIT cell cytotoxic responses to NTHi(115)	**Human:** •T cell responses correlate with better disease control against *H. influenzae* in patients with bronchiectasis(116)	**Human:** •IgG against Elongation-factor thermo unstable may play a role in protection against NTHi(117) •HMW1 and HMW2 specific antibodies are capable of mediating opsonophagocytic killing of NTHi(118)	**Human:** •Patients with cystic fibrosis who have chronic NTHi infections, had elevated levels of IL-8 and CXCL1(119) •NTHi activation of T cells results in a pro-inflammatory response that causes fibrosis in COPD patients(120)
	Animal models: •Peli1$^{-/-}$ mice have an enhanced capacity to recruit neutrophils to airways following infection with NTHi, as well as an enhanced bacterial clearance capacity(121)	**Animal models:**	**Animal models:** •Pulmonary immunisation of mice with heat killed NTHi generated broad protection against heterologous strains through a Th17 T cell response(122)	**Animal models:** •Phosphorylcholine specific IgM and CRP binding to NTHi enhances complement-mediated killing(123) •NTHi uptake of sialic acid can prevent IgM binding and evasion of complement-mediated killing(124)	**Animal models:** •Mice with chronic NTHi had elevated levels of a range of cytokines, increased neutrophilic infiltration and $CD4^+$, all of which is associated with airway remodelling(119)
K. pneumoniae	**Human:** •*K. pneumoniae* is able to inhibit NET formation and the release of primary granules, evading neutrophil action(125) •*K. pneumoniae* can inhibit ROS generation in neutrophils(126) •IL-1β, IL-23 and TNF-α production by antigen presenting cells varies depends on the strain of *K. pneumoniae*(127)	**Human:** •DC recruit NK cells in a CCR5-dependent pathways during *K. pneumoniae* infection(128)	**Human:** •IL-17 production by $CD4^+$ T cells varies depending on the strain of *K. pneumoniae*(127)	**Human:** •Rabbit antibodies enhanced human serum and neutrophil killing of *K. pneumoniae*(129) •Human antibodies generated against *K. pneumoniae* LPS also recognize non-*K. pneumoniae* LPS(130)	**Human:**
	Animal models: •IL-10 producing monocyte myeloid derived suppressor cells in mice enhance *K. pneumoniae* clearance(131) •*K. pneumoniae* can modulate mouse neutrophil cell death pathways and impair efferocytosis(132)	**Animal models:** •MR1$^{-/-}$ mice have lower survival rates compared to MAIT cell replete mice(42) •NK cell mediated type I IFN signalling is required for bacterial control and clearance in mice(133) •γδ T cells mediate early production of INF-γ and TNF-α during acute *K. pneumoniae* infection in mice(134)	**Animal models:** •Following immunisation with heat killed *K.pneumoniae*, a population of protective lung $CD4^+$ T_{RM} is established from effector T_H17 cells in mice(135) •Mice infected intra-tracheally with *K.pneumoniae* generated a strong T_H1 response in the lung(136)	**Animal models:** •The anti-capsular antibodies17H12 and 8F12 provide broad protection against *K.pneumoniae*(137) •Vaccination of mice with OmpK17 and OmpK36 resulted in strong IgG1 response, and protection against *K.pneumoniae* challenge(138)	**Animal models:**

M. tuberculosis (including BCG)	**Human:** •Monocyte derived IL-1β and IL-6 appear to enhance T_H17 responses(139)	**Human:** • TB reactive MAIT cell frequencies are reduced in peripheral blood whilst bolstered in lung tissue in patients with active TB(38) • MAIT cells can be found in pleural fluid of patients with TB and had a greater IFN- γ, IL-17F and granzyme B response compared to MAIT cells from peripheral blood(140) • TB latency is associated with increased MAIT and CD1d-resticted T cells(141)	**Human:** • Human PBMC in vitro infection model demonstrated that depletion of T_{reg} cells results in a skew away from a T_H1 to a T_H2 milieu, an environment that allows for better survival of *M. tuberculosis*(142) • $CD4^+CD161^+$ T cells act rapidly to inhibit intracellular *M. tuberculosis* on initial exposure via perforin and IFN-γ(143) • T_{RM} from TB infected patients secreted IL-2, IFN-γ and TNF-α when exposed to *M. tuberculosis*(64)	**Human:** • B cells in the pleura of TB infected patients are a source of type I IFN during active disease(144) • Stimulation of B cells from patients with latent TB produce IL-1β, IL-10, IL-17, IL-21 and TNF-α(145)	**Human:**
	Animal models: • Stimulation of TLR9 on antigen presenting cells generates type I IFN signalling dependant protection against *M. tuberculosis* in mice(146) • Mouse RdfE-matured dendritic cells are capable of inducing anti-mycobacterial T_H1 and T_H17 cell responses(147) • Costimulation of CD40 by dendritic cells in mice assists with the induction of antigen specific T_H17 cell responses(148)	**Animal models:** • Mouse MAIT cells inhibit intracellular BCG growth via IFN-γ secretion. $Mr1^{-/-}$ mice suffer higher bacterial burden compared to WT mice during early low dose BCG infection(37) • Intravenous BCG administration in non-human primates generated a large population of MAIT cells at the time of *M. tuberculosis* which is associated with near sterilizing protection(90) • $CD27^+$ NK cells are associated with protection in latent *M. tuberculosis* infection of non-human primates(149) • Glucose monomycolate specific CD1 restricted T cells following intravenous BCG vaccination in non-human primates provide close to sterilizing protection against *M. tuberculosis*(150)	**Animal models:** •Intratracheal adoptive transfer of T_{RM} provided protection against *M. tuberculosis* in naïve mice(60) •An infection dose dependant differentiation of T cell responses occurs in non-human primate lungs at early stages of tuberculosis infection(151)	**Animal models:** • IL-4 sensitive B cells are associated with increased lung pathology, decreased lung IgA and reduced protection in a murine model of chronic *M. tuberculosis* infection(152) • IL-6 does not appear to have a significant effect on T_H17 responses during *M. tuberculosis* infection(153)	**Animal models:** •Inhibition of the integrated stress response prevented necrosis of lung granulomas in *M. tuberculosis* infection of $sst1^S$ mice, reducing bacterial burden(154) •Mice infected with *Mtb* mutant Δ*mmpl7* are associated with increased iBALT formation(155)
P. aeruginosa	**Human:** • Exposure to *P. aeruginosa* flagellin stimulates 'innate immune memory' in bronchial epithelial cells through epigenetic changes(156) • In vitro administration of IL-17C to nasal epithelium cell culture infected with *P. aeruginosa* reduced bacterial growth(157)	**Human:** • Low MAIT cell counts are correlated with *P. aeruginosa* infection in patients with cystic fibrosis(158)	**Human:** • Elevated levels of T_H17 and T_H2 responses may indicate impending *P. aeruginosa* infection in patient with cystic fibrosis(159) • Chronic *P. aeruginosa* infection is associated with an age-dependant decline in T_{reg} cell function in patients with cystic fibrosis(160) • *P. aeruginosa* specific T_H22 cells are induced in patients in cystic fibrosis(161)	**Human:** • Individuals with low anti-PcrV titers and chronic *P. aeruginosa* infection may have a poorer outcomes(162) • Impaired local immunity and reduced IgA-mediated responses against *P. aeruginosa* may allow for recurrent infection in severe COPD(163) • sIgA is correlated with the carriage status of *P. aeruginosa* in patients with cystic fibrosis(164)	**Human:** • Peribronchial lymphoid structures found in the lung tissues of cystic fibrosis bronchiectasis patients are attributed to *P. aeruginosa* infection(165)
	Animal models: • Airway epithelial cell DNA methyltransferase 3b dampens the mucosal response to *P. aeruginosa* flagellin(166) • Experimental infection of mice with *P. aeruginosa* demonstrates that alveolar macrophages rapidly phagocytose bacteria in order to avoid unnecessary neutrophil recruitment(167) • Type I IFN mediated NETosis promotes *P. aeruginosa* persistence in the lung through biofilm formation(168)	**Animal models:** • IL-17 production by γδ T cells may improve immunity against *P. aeruginosa* through innate and humoral responses in mice(169)	**Animal models:** • $CD4^+$ T cells expressing IL-17A are significantly increased in the lungs of mice vaccinated with a live attenuated strain of *P. aeruginosa* that protected against lethal pneumoniae(170) • Suppression of the pulmonary T_H17 response in the lungs of mice with chronic *P. aeruginosa* ameliorated neutrophil mediated inflammation(171)	**Animal models:** • Anti-PcrV IgG appear to reduce inflammation during chronic respiratory *P. aeruginosa* infection in mice(172)	**Animal models:** • iBALT appear to have a role in modulating the local immune response to chronic *P. aeruginosa* infection in the lungs of rats(173) • IL-8 expression by murine bronchial epithelial cells enhanced protection against *P. aeruginosa*; however it also resulted in lung remodelling affecting physiological functions(174)
C. pneumoniae	**Human:** • *C. pneumoniae* reduces TRAF-3-dependant innate responses during epithelial cell infection(175)	**Human:**	**Human:** • *C. pneumoniae* may be capable of causing local T cell suppression(176) • Convalescent patients maintained a population of $CD4^+$ central memory T cells that generated IFN-γ and IL-2 upon stimulation with *C. pneumoniae*(177)	**Human:** • Individuals lacking a strong T cell IFN- γ and IL-2 response to *C. pneumoniae* maintained high specific IgG reactivity to *C. pneumoniae*(177)	**Human:** • Stimulation of PBMCs from asthmatic patients with *C. pneumoniae* resulted in the generation of T_H2 responses and IgE production(178) • Stimulation of PBMCs from asthmatic patients with *C. pneumoniae* resulted in the generation of a strong IFN-γ response(179)
	Animal models: • Plasmacystoid dendritic cells are vital in ensuring a balanced T cell inflammatory response is generated in mice during *C. pneumoniae* infection(180) • Mast cell recruitment of immune cells during *C. pneumoniae* infection in mice resulted in increased bacterial replication(181)	**Animal models:** • NK cells skew T cell responses towards a protective T_H1 response during *C. pneumoniae* infection in mice by regulating dendritic cell function(182)	**Animal models:** • $CD8^+$ T cells mediate protection against *C. pneumoniae* reinfection in mice(183) • IFN-γ appears vital in protection against *C. pneumoniae* reinfection in mice(184)	**Animal models:** • A large increase of B cell numbers occurs in the lungs of mice during primary infection with *C. pneumoniae*(185)	**Animal models:** • Infection of mice with *C. pneumoniae* resulted in iBALT formation, chronic inflammation and fibrosis of the lungs(186) • Vascular smooth muscle cell migration is stimulated through TLR-2 related pathways during *C. pneumoniae* infection(187)
	Human:	**Human:**	**Human:**	**Human:**	**Human:**

M. pneumoniae (including mycoplasma spp)	• Bronchial epithelial cells infected with *M. pneumoniae* stimulated the production of GM-CSF, in turn activating neutrophil production of myeloperoxidase(188) • Children suffering from *M. pneumoniae* pneumonia had significantly increased levels of IL-10 but not IL-4 in their serum(189)	• Patients with *M. pneumoniae* had significantly higher frequencies of circulating IL-17 producing cells than healthy patients' γδ T cells(190)	• *M. pneumoniae*-specific IFN-γ secreting $CD4^+$ effector memory T cells are correlated with the severity of *M. pneumoniae* pneumonia(191) • Patients with *M. pneumoniae* had significantly higher frequencies of circulating T_H17 cells than healthy patients(190) • Higher T_H17/T_{reg} ratio is associated with refractory *M. pneumoniae* pneumonia(192)	• Antibody secreting cells are detected in circulation up to 6 weeks following symptom onset of *M. pneumoniae* pneumonia in children(193)	• *M. pneumoniae* P1 protein acts as an allergen and induced IgE production(194)
	Animal models: • *M. pneumoniae* can evade NET-mediated killing through the production of a neuclease called Mpn491(195) • MyD88-NFκB pathway in macrophages is required for clearance of *M. pneumoniae* from the lungs of mice(196)	**Animal models:**	**Animal models:** • $CD25^+$ T_{reg} cells suppress the damaging effects of T_H2 responses, whilst promoting IL-17 and IFN-γ during mycoplasma pneumonia in mice(197) • Depletion of $CD8^+$ T cells in mice infected with mycoplasma resulted in more severe disease(198) • $CD4^+$ T cell depletion in mice infected with mycoplasma resulted in less severe disease(198)	**Animal models:** • Protection mediated by antibodies in mice is largely attributed to IgG against *M. pneumoniae* proteins rather than glycolipids(199) • Whilst antibody responses aid with clearance of *M. pneumoniae* from the lower respiratory tract in mice, they appear to have a limited role in the upper respiratory tract(200)	**Animal models:** • Community-acquired respiratory distress syndrome toxin produced by *M. pneumoniae* induced asthma-like histological changes in the airways of baboons(201)

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Abbreviations

B_{RM} cells – resident memory B cells

cDCs – conventional dendritic cells

COPD – chronic obstructive pulmonary disease

DCs – dendritic cells

DURTs – donor-unrestricted T cells

iBALT – inducible bronchus associated lymphoid tissue

IgA – immunoglobulin A

IgG – immunoglobulin G

IgM – immunoglobulin M

MAIT cells – mucosal-associated invariant T cells

mDCs – monocyte-derived dendritic cells

NETs – neutrophil extracellular traps

NKT cells – natural killer T cells

NO – nitrous oxide

PAMPs – pathogen-associated molecular patterns

PRRs – pattern recognition receptors

RAMD – repair-associated memory depots

ROS – reactive oxygen species

TB – tuberculosis

T_{EM} cells – effector memory T cells

T_{RM} cells – tissue resident memory T cells

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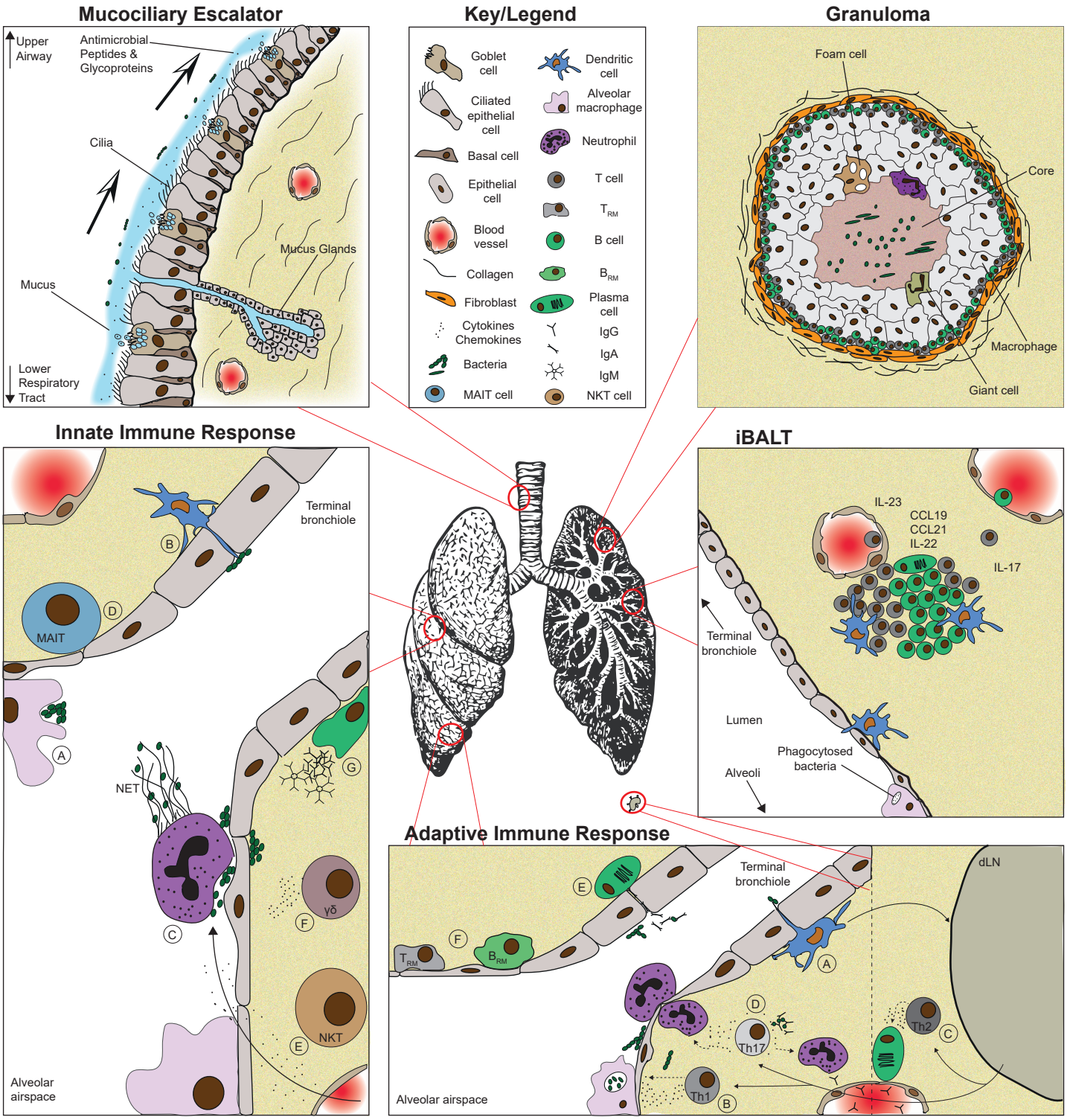
FIGURE LEGEND

Fig. 1. Generic immune responses utilised during bacterial lung infections. The mucociliary escalator (upper-left of the figure) is the first line of defence against bacterial infections of the lungs. The ciliated pseudocolumnar epithelium of the upper respiratory tract secretes mucus via mucus glands and goblet cells, which traps foreign material including bacteria. This mucus is then swept towards to the larynx, stimulating a cough reflex to eliminate trapped foreign materials. A range of antibacterial molecules are also secreted to inhibit bacterial growth. Among innate immune responses (lower left of the figure), bacteria that escape into the lower respiratory tract are met with a wide range of innate immune responses at the terminal bronchioles and alveolar airspaces. The epithelial cells can initiate inflammatory responses once bacterial invasion is detected through several pathways including PRRs. (A) Alveolar macrophages are poised to phagocytose local bacteria. Once macrophages are overwhelmed, (B) DCs can aid in phagocytosis. (C) Neutrophils are recruited to sites of high bacterial load via a range of chemical signals. Neutrophils assist with bacterial clearance through phagocytosis, pathogen killing through granule-mediated mechanisms and NETosis. However, neutrophils can also cause significant immunopathology. (D–F) DURTs including MAIT cells and CD1-resticted T cells, as well as γδ T cells are capable of initiating rapid effector functions such as cytokine release and cytolysis. (G) Non-specific immunoglobulin produced by B cells and from previous immune responses can also assist with opsonophagocytosis during early infection. Among adaptive Immune response (lower-right of the figure), (A) DCs play a critical role in antigen presentation. Once bacteria are engulfed and antigens are processed, DCs can migrate to draining lymph node (dLN) to initiate and skew adaptive immune responses. (B) T_H1, (C) T_H2 and (D) T_H17 responses may take place depending on the type of inflammatory/signalling pathways induced. $CD8^+$ T cell responses play a significant role in cytolysis of infected cells (not shown in figure). (E) B cells can also be activated to differentiate into plasma cells that produce specific immunoglobulin against bacteria. In particular, IgA is produced to protect against bacterial invasion at the epithelial surface through a process called ‘immune-exclusion’. (F) Resident T and B cells can be generated to remain poised in pathogen-cognisant lung tissue to serve in an immunosurveillance role and mount rapid secondary responses

when needed. However, these cells may be shorter-lived than their counterparts in other barrier tissues because of the immunosuppressive landscape of the lung under steady-state conditions. In iBALT (middle-right of the figure), in some cases, B and T cells congregate in bronchi to form highly organised structures that resemble secondary lymphoid organs. These structures have been identified as a source of continued antigenic stimulation and maintain a local pool of pathogen-specific memory cells, enhancing protection. However, they have also been identified in several disease states of the lung. In situations where the pulmonary immune system is unable to clear bacteria from the lung, a granuloma that contains the infection may be formed (upper-right of the figure). These structures require the aggregation and interaction of macrophages, neutrophils, B cells, T cells and fibroblasts in order to ‘wall off’ bacteria to prevent dissemination. Although granulomas can be effective at maintaining latency of infection, immune dysfunction can result in the breakdown of the structure and allow for re-activation of disease.


Mucociliary Escalator
Upper Airway
Antimicrobial Peptides & Glycoproteins
Cilia
Mucus Glands
Mucus
Lower Respiratory Tract
Key/Legend
Goblet cell
Ciliated epithelial cell
Basal cell
Epithelial cell
Blood vessel
Collagen
Fibroblast
Cytokines Chemokines
Bacteria
MAIT cell
Dendritic cell
Alveolar macrophage
Neutrophil
T cell
T_{RM}
B cell
B_{RM}
Plasma cell
IgG
IgA
IgM
NKT cell
Granuloma
Foam cell
Core
Macrophage
Giant cell
Innate Immune Response
Terminal bronchiole
MAIT
NET
γδ
NKT
Alveolar airspace
iBALT
IL-23
CCL19
CCL21
IL-22
IL-17
Terminal bronchiole
Lumen
Phagocytosed bacteria
Alveoli
Adaptive Immune Response
Terminal bronchiole
dLN
Th1
Th2
Th17
Alveolar airspace