Pulmonary immune response to *Streptococcus pneumoniae*

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Summary

***Streptococcus pneumoniae* is a common cause of respiratory infection associated with significant morbidity and mortality. The immune response to *S. pneumoniae* is complex, multi-layered and incompletely understood. The lung has non-specific defence mechanisms that include cellular and secreted components. The initial response to bacteria that adhere to or breach the epithelium is mediated by the innate immune system and recognition of non-specific motifs common to many pathogens. This stimulates the inflammatory response and subsequently the development of the more specific adaptive immune system. Better understanding of the immune response to *S. pneumoniae* will underpin future progress in both the prevention of infection and adjuvants to existing treatments.**

KEY WORDS: Streptococcus pneumoniae; immunology.

Introduction

Streptococcus pneumoniae is the commonest cause of pneumonia and is thought to be responsible for over a million deaths per year, particularly in children and the elderly (1). Despite effective antibiotics *S. pneumoniae* accounts for 33% of vaccine preventable deaths due to pneumonia in children worldwide (2). Community acquired pneumonia (CAP) is also associated with substantial morbidity

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and high economic burden, costing 10.1 billion euros per year in Europe (3). Host factors that are known to increase the risk of pneumococcal infection include immune deficiency (e.g. HIV infection, immunoglobulin deficiencies, complement deficiencies, myeloma, post organ transplant), splenic dysfunction (e.g. asplenia, sickle cell disease), and chronic organ dysfunction (e.g. liver cirrhosis and chronic lung disease) (4). The extremes of age, smoking, alcohol abuse, and influenza also increase the risk of contracting disease. Given the importance of this bacterium in lung disease, understanding the pulmonary host immune response is essential for the design of more effective therapeutic interventions.

S. pneumoniae biology

S. pneumoniae is a gram positive lancet shaped bacterium that usually grows as diplococci. It is a nasopharyngeal commensal, a site where it does not cause significant pathology. Rates of colonisation are particularly high amongst nursery age children, but reduce to approximately 10% in healthy adults (5). *S. pneumoniae* reaches the lower respiratory tract after microaspiration and are much more likely to cause infection when pulmonary host defence mechanisms are disrupted. Invasion through epithelial layers either locally from the nasopharynx or from the lung during pneumonia can lead to septicaemia.

> **S. pneumoniae needs to adhere to respiratory epithelium, so infection is mediated by the interactions of bacterial and host cell surface structures.**

All virulent strains of *S. pneumoniae* express a polysaccharide capsule that surrounds a cell wall consisting of peptidoglycan and teichoic acid. The capsule's primary function is to inhibit opsonophagocytosis by the host immune system (6). There are over 90 different serotypes, classified by the composition of the oligosaccharide makeup of the capsule. Duration of colonisation, invasiveness, and case-fatality rates vary with capsular serotype, for example serotypes 3, 6A, 6B, 9N, 19F, 23F, 31 are overrepresented in fatal cases of infection (7, 8). Other important *S. pneumoniae* virulence factors are a range of secreted and cell wall-associated proteins. These include the toxin pneumolysin that has pleiotropic effects on the host immune response including cell lysis, complement activation, inhibition of neutrophil function, and increased inflammation, as well as cell wall proteins that inhibit opsonophagocytosis. In addition, to achieve

successful infection, *S. pneumoniae* needs to adhere to respiratory epithelium, which is mediated by the interactions of bacterial and host cell surface structures. These include hydrophobic and electrostatic surface characteristics contributing to physiochemical interactions, and *S. pneumoniae's* ability to bind to N-acetyl-galactosamine on resting epithelium (9), the ability of the cell wall protein PspC (pneumococcal surface protein C) to bind to the laminin receptor expressed on a variety of host cell types (10), and cell wall phosphocholine which binds to the platelet activating factor receptor (11). Bacterial/host cell interactions also stimulate active bacterial translocation across epithelial and endothelial layers to reach the blood or central nervous systems (12).

Host defence mechanisms

The pulmonary host defence mechanisms to *S. pneumoniae* are multi-layered and involve many complex interactions between separate components of the immune system. Initial physical pulmonary defences include the mucociliary escalator and the integrity of the pulmonary epithelium. Mucus entraps pathogens, inhibiting adherence of the bacteria to the underlying epithelium, and the action of cilia moves the entrapped pathogens out of the lung. An intact epithelium prevents bacterial adhesion to extracellular matrix and penetration into the lung interstitium and eventually the blood. Damage to these physical defences through viral infection of the epithelium (13) or inhibition of mucociliary function by cigarette smoke (14) increase the ability of invading bacteria to establish infection within the lungs. *S. pneumoniae* is a classic extracellular pathogen that replicates and persists mainly outside of host cells; hence the killing of *S. pneumoniae* that manage to evade physical defences is largely mediated by opsonophagocytosis and secreted proteins and peptides in epithelial lining fluid. To assist bacterial clearance*, S. pneumoniae* reaching the lungs stimulate an inflammatory response that recruits and activates immune cells to the lungs, including phagocytes. Additionally, an acute phase response is initiated allowing soluble serum mediators such as complement to enter the lung compartment following an increase in vascular permeability. Previous colonisation means that as well as the innate immune response all humans have some degree of adaptive immunity that can recognise *S. pneumoniae* and potentially assist bacterial clearance from the lungs. The most important aspects of the innate and adaptive immune response will be discussed separately below, although in reality they act in concert as an integrated system for prevention and control of *S. pneumoniae* persistence within the lung.

> **Damage to physical defence mechanisms through viral infection of the epithelium or inhibition of mucociliary function by cigarette smoke increase the ability of invading bacteria to establish infection within the lungs.**

Innate immune response

Epithelial cells and surfactant

Type II pneumocytes secrete antibacterial products into alveolar lining fluid which assists with bacterial killing. These include: (a) lactoferrin, which sequesters free iron required for bacterial growth and oxidises bacterial cell membranes; (b) lysozyme, which breaks down peptidoglycan in bacterial cell walls; and (c) a wide range of antimicrobial peptides (e.g. defensins and cathelicidin) that form pores in bacterial cell surfaces thereby lysing invading pathogens. The efficacy of these soluble components of mucosal defences against *S. pneumoniae* has in general been poorly defined. *S. pneumoniae* is relatively resistant to lactoferrin as it produces a cell wall protein, pneumococcal surface protein A (PspA), that binds to lactoferrin and blocks its activity (15). Similarly, while lysozyme can restrict the growth of *S. pneumoniae,* modifications to its cell wall structure render it relatively resistant to degradation (16, 17). However, the antimicrobial peptide β-defensin-2 does cause *S. pneumoniae* cell lysis and can act synergistically with lysozyme in controlling bacterial numbers (17). As well as secreting soluble antibacterial factors, epithelial cells express pattern recognition receptors (PRR) that recognise conserved molecular patterns found on the surface of microbial pathogens, including *S. pneumoniae*. Activation of epithelial PRRs can contribute towards the inflammatory response to *S. pneumoniae* (18). However, epithelial pro-inflammatory responses can be indirectly increased by macrophages (19). This suggests that alveolar macrophages are likely to be the cell type that primarily recognise *S. pneumoniae*, and then stimulate the alveolar epithelium by paracrine or juxtacrine mechanisms to amplify the inflammatory response, and attract required additional cells such as neutrophils to the site of infection.

Surfactant protein A & D (SP-A & D respectively) are constitutively synthesized and secreted into alveolar lining fluid by type II pneumocytes and non-ciliated bronchial epithelial cells. SP-A & D bind to exposed mannose and glucose residues on the surface of bacteria, and this leads to agglutination of pathogens, inhibition of microbial growth, and increase recruitment of phagocytes. SP-A has also been shown to be an opsonin that increases alveolar macrophage phagocytosis of *S. pneumoniae* (20).

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Alveolar macrophages

Macrophages are tissue resident phagocytes that play an important role in orchestrating innate immune responses to pathogens. Alveolar macrophages make up most of the resident leukocyte population in the alveolar space. The vital role for alveolar macropha-

ges for preventing *S. pneumoniae* pneumonia is suggested by the increased incidence of pneumonia in humans with impaired macrophage function due to exposure to cigarette smoke, viral infection, corticosteroids, welding fumes, or alcohol (21-25). Alveolar macrophages are able to phagocytose foreign material, coordinate an inflammatory cytokine response to infection and act as antigen presenting cells. They kill pathogens by a variety of mechanisms, including the generation of oxidative species and serine proteases. Despite their important role as first-line of cells in pulmonary host defence, alveolar macrophages exhibit less phagocytic activity and respiratory burst than other types of macrophages, and are less likely to stimulate T cells; these phenotypes help to promote tolerance to innocuous antigens that reach the respiratory tract. In addition alveolar macrophages, by secreting TGFβ and prostaglandins may drive differentiation of T cells into a regulatory phenotype that suppress inflammation (26). This reflects the important role alveolar macrophages have for maintaining homeostasis within the alveolar compartment, preventing continuous or uncontrolled inflammatory responses within the lung to the almost constant exposure to external antigens that occurs, in order to avoid the deleterious effects of inflammation on the delicate lung tissue.

Despite their relative anti-inflammatory resting state, alveolar macrophages express an array of PRRs including indirect phagocytic receptors such as Fc receptors for immunoglobulins and complement receptors for the activated complement components C3b and iC3b bound to the surface of invading pathogens, direct phagocytic receptors including lectins (dectin-1 and DC-SIGN), and scavenger receptors (SR-A and MARCO), and direct non- phagocytic receptors such as toll-like receptors (TLR). Through phagocytic receptors alveolar macrophages can ingest and kill invading bacteria, and evidence from murine models suggest they can rapidly clear low numbers of *S. pneumoniae* from the lungs (27). Hence, alveolar macrophages can deal with low levels of infection by phagocytosis. However, when large numbers of *S. pneumoniae* reach the lung they overwhelm alveolar macrophage mediated clearance. In response, alveolar macrophages initiate an inflammatory response through the release of pro-inflammatory cytokines including TNF, IL6, and IL-1β and chemokines (19, 28), thereby recruiting additional mechanisms of microbial clearance, such as neutrophils, to the lung. Conversely, anti-inflammatory cytokines released by alveolar macrophages are necessary for resolution of inflammation (29). Furthermore, following neutrophil clearance of infection, macrophages also remove apoptotic neutrophils thereby assisting with resolution of inflammation (29, 30); failure of alveolar macrophages to clear neutrophils results in neutrophil necrosis, the release of reactive oxygen species and proteases, and subsequent tissue injury and inflammation (31). The ability of alveolar macrophages to limit inflammation for the majority of the time, but elicit an inflammatory response to a significant invasion by pathogens may be mediated by their close relationship with alveolar ep-

ithelium. This allows negative regulators present on the alveolar epithelium (e.g. the surface proteins CD200 on CD200R) to inhibit alveolar macrophage-mediated inflammation, but the inhibitory effect is lost after epithelial damage.

Neutrophils

Neutrophils or polymorphonuclear leukocytes are the most abundant leukocyte population and are usually the initial cells recruited to sites of injury and infection. Although there are very few neutrophils present on the epithelial surface or interstitium of normal lungs, the pulmonary capillary bed contains a significant reservoir of intravascular neutrophils that are rapidly recruited to the alveolar space during poorly controlled bacterial infection. Recruited neutrophils play a crucial role in host defence to bacterial pathogens, as demonstrated by an increased incidence of *S. pneumoniae* pneumonia when neutrophil function is impaired (e.g. Chediak-Higashi syndrome where there is failure of phagolysosome formation and reduced lysosomal degranulation) or neutrophil numbers are reduced (32, 33). On the other hand, neutrophilic inflammation is also implicated in tissue damage that can result in lung injury and the develop-

> **Recruited neutrophils play a crucial role in host defence to bacterial pathogens, as demonstrated by an increased incidence of S. pneumoniae pneumonia when neutrophil function is impaired or neutrophil numbers are reduced.**

ment of the acute respiratory distress syndrome. Additionally, they have been implicated in the development of bacteraemia, with some murine studies suggesting that neutropenia is associated with reduced bacteraemia and prolonged survival (19, 34).

Neutrophils primarily engage with pathogens via phagocytosis followed by the formation of phagolysosomes and intracellular killing. Neutrophil phagocytosis of *S. pneumoniae* is highly dependent on recognition of the bacteria by proteins of the complement system or by specific antibody (6, 35). Neutrophils also produce neutrophil extracellular traps (NETs) by extruding their chromatin and DNA to form extracellular filaments that entrap pathogens and expose them to antimicrobial compounds. However, *S. pneumoniae* is relatively resistant to NETs by virtue of its capsule and expression of surface endonucleases that degrade DNA (36). *S. pneumoniae* is also resistant to the main mechanism of bacterial killing by neutrophils, the NADPH oxidase-dependent oxidative burst that generates reactive oxygen. As a result, humans with chronic granulomatous disease, who fail to produce ROS, don't have excessive rates of *S. pneumoniae* infection. Instead, *S. pneumoniae* killing by neutrophils is mediated by azurophilic granules that contain the serine proteases cathepsin G and neutrophil elastase which degrade bacterial proteins (37, 38), defensins (17), and bactericidal permeability-increasing protein (39).

Table 1 - PRRs and their potential *S. pneumoniae* ligand/activating molecule.

PRR	Where PRR is expressed	*S. pneumoniae* ligand	Consequences of activation
TLR 2	Leucocyte and epithelial cell surface	Lipoproteins Cell wall constituents?	Proinflammatory cytokine transcription
TLR 4	Leucocyte and epithelial cell surface	Pneumolysin?	Proinflammatory cytokine transcription
TLR 9	Leucocyte endosomes	Unmethylated CpG DNA fragments	Proinflammatory cytokine transcription Type 1 IFN transcription
NOD 2	Leucocyte cytoplasm	Cell wall muramyldipeptide	Proinflammatory cytokine transcription
NLRP 3	Cytoplasm	Pneumolysin ? indirect	Activation of pro IL1β and IL18
DC-SIGN / SIGN-R1	Macrophages and dendritic cells (marginal zone of spleen)	Capsular polysaccharide?	Increased phagocytosis Complement activation? Improved natural IgM production?
Unknown	Cytoplasm	Bacterial DNA	Activation of STING to induce Type 1 IFN transcription
MARCO	Macrophages	Unknown	Increased phagocytosis Amplification of other PRR signalling
Scavenger receptors	Macrophages	Unknown	Increased phagocytosis
SP-A & SP-D surfactant proteins	Release into epithelial lining fluid by Type II pneumocytes and Clara cells	Surface mannose & glucose residues	Increased phagocytosis

Pattern recognition receptors

The host immune system first recognises *S. pneumoniae* via PRR expressed in epithelial cells and macrophages within the respiratory tract (Table 1). These receptors recognise pathogen associated molecular patterns (PAMP) such as components of the pneumococcal cell membrane (lipoproteins) (40), cell wall (peptidoglycan and lipoteichoic acid), and also recognise damage associated molecular patterns (DAMPs e.g. the HGMB1 and S100 host proteins) released by endogenous cells as a result of tissue damage (Figure 1). PRRs are often situated on cell surface membranes, but are also present on endosomes and as such are in position to recognise PAMPs released into phagolysosomes by bacterial degradation. The most important PRRs for alveolar macrophage interactions with *S. pneumoniae* are described in more detail below.

Toll-like-receptors

The most important non-phagocytic PRRs for *S. pneumoniae* belong to the Toll-like-receptors (TLR) family of single, membrane-spanning, non-catalytic proteins. TLR 1, 2, 4, 5, and 6 are situated on the cell surface membrane and the rest reside on endosomes. When they recognise PAMPs they activate cell signalling pathways by a number of adaptor molecules (MyD88, TRAP, TRIF, and MAL) that culminate in altered gene transcription. Children with interleukin-1 receptor-associated kinase 4 (IRAK4) and similar deficiencies that affect TLR intracellular signalling pathways have impaired macrophage pro-inflammatory responses and high incidences of often lethal *S. pneumoniae* infections (41).

> **The absence of TRL9 results in reduced survival during S. pneumonia infection, probably because TLR9 activation improves bacterial phagocytosis by alveolar macrophages.**

TLR 2 forms heterodimers with TLR6 to recognise diacylated lipoproteins, proteins that are covalently anchored to the bacterial cell membrane, including *S. pneumoniae* (40, 42, 43). The importance of TLR2 in induction of the inflammatory response to *S. pneumoniae* has been demonstrated in a mouse model of infection with TLR2$^{-/-}$ mice having decreased cytokine (e.g. IL1β, IL6, and KC, the mouse homologue of CXCL8) responses to *S. pneumoniae*. Recent data suggests that lipoproteins are important TLR2 ligands that are required for IRAK4 mediated protective inflamma-
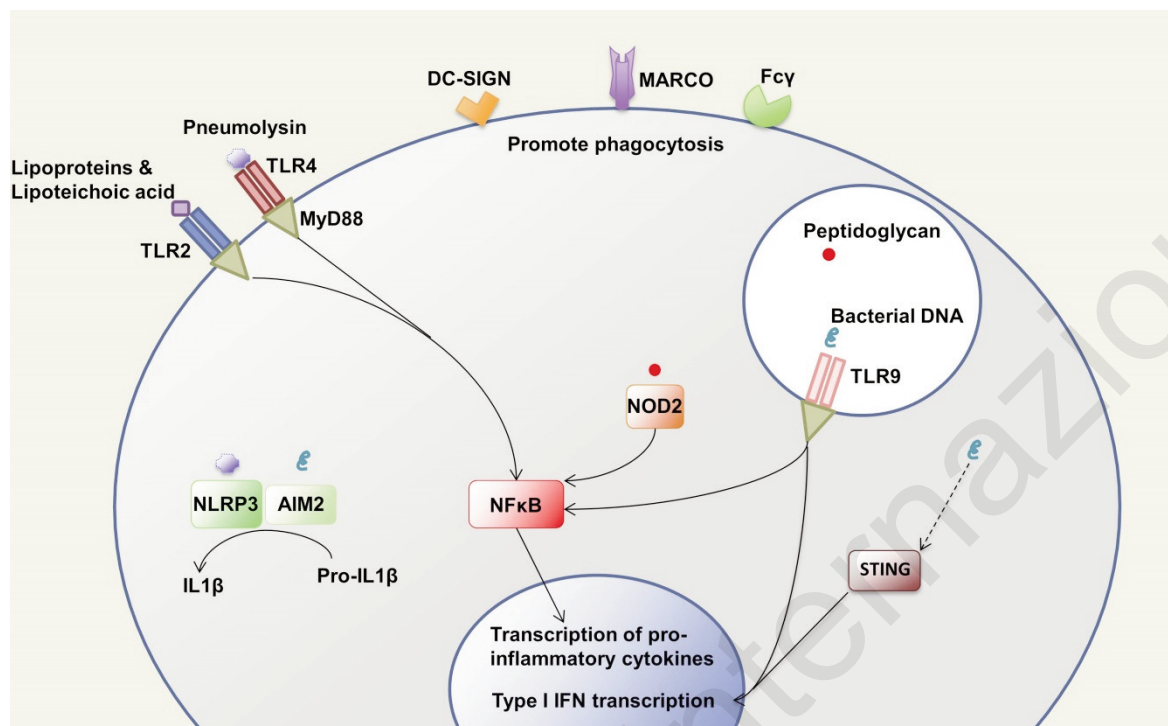
Figure 1 - PRR that interact with *S. pneumoniae*.
Several PRR (pattern recognition receptors) recognise *S. pneumoniae*. Non-opsonic phagocytic receptors like DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) and MARCO (Macrophage receptor with collagenous structure) recognise components of *S. pneumoniae* and induce its phagocytosis. Opsonic receptors like Fcγ receptors recognise antibody that has bound *S. pneumoniae*, and again induce phagocytosis. TLRs (Toll like receptors) are cell surface and endosomal receptors that trigger intracellular signalling cascades to stimulate transcriptional events. TLR2 bind lipoproteins and TLR4 pneumolysin to induce NFκB translocation into the nucleus and the transcription of pro-inflammatory cytokines like TNF, IL1β, and IL6. Cytoplasmic PRR like NOD2 (Nucleotide-binding oligomerization domain-containing protein 2) recognise peptidoglycan from *S. pneumoniae* cell wall and also activate NFκB. Bacterial DNA is recognised directly by TLR9 and indirectly by cytoplasmic signalling molecules like STING (Stimulator of interferon genes). Signalling cascades downstream of these events induce type I interferon transcription. The inflammasome components NLRP3 (NOD-like receptor family, pyrin domain containing 3) and AIM2 (Absent in melanoma 2) are activated by pneumolysin dependent mechanisms and bacterial DNA respectively to modify pro-cytokines into their secreted functional forms.

tory responses in humans (40).
TLR4 is usually stimulated by lipopolysaccharide from Gram negative bacterial cell walls, but may also be important for inflammatory response to *S. pneumoniae* perhaps through direct stimulation by the toxin pneumolysin (40, 44). TLR9 recognises the unmethylated CpG motifs of prokaryotic organisms and downstream signalling causes transcription of pro-inflammatory cytokines and type I interferons. The absence of TLR9 in mice results in reduced survival during *S. pneumoniae* infection, probably because TLR9 activation improves bacterial phagocytosis by alveolar macrophages (45).
There is built-in redundancy within the TLR system, with greater effects on morbidity and mortality when TLR signalling adaptor molecules are affected than after deletion of individual TLR abnormalities. For example absence of MyD88, an adaptor protein that is common to the function of all TLRs except for TLR3, is associated with worse outcomes of *S. pneumoniae* in-

fection in mouse models of CNS infection, nasopharyngeal colonisation, pneumonia and sepsis than individual absence of TLR2 or TLR4 (46, 47). Additionally, IRAK4 deficiency results in a blunted pro-inflammatory cytokine secretion profile and increased mortality in both mice and humans during *S. pneumoniae* infection (41, 48), whereas TLR2 deficiency alone has minimal effects on mortality in mouse models of pneumonia (49).

Cytosolic PRRs

NOD like receptors (NLRs) are a group of intracellular, primarily cytosol-based PRRs (50). NOD2 is expressed on leucocytes and epithelial cells (51) and recognises muramyl dipeptide released from the *S. pneumoniae* cell wall during lysozyme-dependent digestion of bacteria in the phagolysosome (52), causing NFκB activation and subsequent pro-inflammatory

gene transcription (53). Within macrophages, cytosolic protein complexes called inflammasomes also promote inflammation by activating the cytokines IL1β and IL18. NLRP3, a key protein of the inflammasome, is activated by pneumolysin and cell wall constituents released by *S. pneumoniae* (54-56), causing greater release of IL1β. There is also evidence that cytoplasmic receptors can recognise *S. pneumoniae* DNA to elicit a type I IFN response. Type I IFN is usually associated with immunity to viral infection but seems to have a significant but poorly understood role in improving innate immune responses against *S. pneumoniae*, perhaps mediated by release of the chemokine RANTES and preventing bacterial translocation across epithelial and endothelial cells (57-59).

Lectins and scavenger non-opsonic phagocytic receptors

Lectins are carbohydrate binding proteins that recognise sugar moieties. Primarily they recognise mannose, fucose, and glucan structures (60). DC-SIGN (dendritic cell-specific ICAM-3 grabbing non integrin) is a human C-type lectin which binds mannose containing ligands and is expressed by macrophages and dendritic cells (61). In mouse models of *S. pneumoniae* infection SIGNR1, the mouse homologue of DC-SIGN, binds to the *S. pneumoniae* capsule, promotes macrophage phagocytosis, and is protective in a mouse model of pneumonia (62, 63). Scavenger receptors are a range of structurally varied PRRs that recognise negatively charged macromolecules through shared similarities in shape and electrostatic charge distribution of their binding sites (64). Alveolar macrophages express the scavenger receptors MARCO (Macrophage receptor with collagenous structure) and/or scavenger receptor A (SR-A). Both receptors have been implicated in pulmonary immunity against *S. pneumoniae* with reduced bacterial clearance in from the lungs in MARCO$^{-/-}$ (65) and SR-A$^{-/-}$ mice (66). Macrophages isolated from MARCO$^{-/-}$ mice show reduced phagocytosis of *S. pneumoniae*, and MARCO activation also appears to amplify the inflammatory response to TLR2 or NOD2 stimulation (67). Further-

more, MARCO appears to be downregulated on alveolar macrophages after influenza infection, contributing to secondary pneumococcal infection (61). Hence, MARCO seems to have a particularly important role for alveolar macrophage mediated immunity to *S. pneumoniae* lung infection.

Acute phase response

The local inflammatory response to *S. pneumoniae* infection in the lungs stimulates a systemic response characterised by increased production of multiple proteins from the liver, many of which play a role in host defence (68) (Table 2). This is termed the acute phase response, and is vital for effective host defence against *S. pneumoniae*. The acute phase response is largely mediated by the cytokine IL6, which is produced in large quantities during *S. pneumoniae* pneumonia and reaches the liver via the circulation (69). Exactly how the acute phase responses boosts immunity is not clear; the large increases in circulating levels of complement proteins, serum amyloid P and C reactive protein associated with the acute phase response should improve recognition of *S. pneumoniae* and therefore neutrophil phagocytosis. Complement is a series of host proteins found in serum, epithelial lining fluid and cell surfaces that form protease cascades. When activated by the host proteins C reactive protein, serum amyloid P, or the complement component C1q binding to the *S. pneumoniae* surface, these protease cascades result in the coating of the bacteria with C3b and iC3b molecules, thereby promoting bacterial phagocytosis via complement receptors (6, 35). The recognition of *S. pneumoniae* antigens by antibody is also a major activator of complement, and is described in more detail in the adaptive immune response section below. In addition induction of fever may inhibit optimal bacterial growth. Furthermore, increased per-

> **The acute phase response is largely mediated by the cytokine IL6, which is produced in large quantity during S. pneumoniae pneumonia and reaches the liver via the circulation.**

Table 2 - Components of the acute phase response that play a role in host defence.

Component	Role in host defence
Complement components: C3,C4,C9, Mannose binding lectin	Opsonisation for phagocytosis Binding of antibody bound to pathogen to effect bacterial agglutination or lysis (35)
C reactive protein	Binds to phosphocholine – increasing agglutination, allowing complement binding and phagocytosis (70)
Serum amyloid P	Binds to negatively charged carbohydrates – allowing complement binding and phagocytosis (71)
Granulocyte Colony Stimulating Factor	Increases granulopoesis and activates mature granulocytes
Coagulation components: e.g. Fibrinogen	Involved in tissue repair and local containment of bacterial infection (72)
α$_1$ protease inhibitor	Inhibit proteolytic enzymes and reduces inflammation-induced damage

meability of the endothelium allows some of these plasma constituents and cellular infiltrate into affected tissues, thereby probably promoting immune clearance within the lung during *S. pneumoniae* infection.

Adaptive immune response

The innate immune response to invading pathogens is generic, able to target conserved microbial structures without the host having had prior exposure to that pathogen. In contrast, the adaptive immune system provides a highly specific immune response that is initiated only after exposure to the pathogen. The adaptive response is highly specific, depending on recognition of specific molecules expressed by pathogens (termed antigens), and provides the optimum protection against an invading pathogen. Generating an adaptive immune response can take days after initial exposure to pathogen, but also generates memory cells, which enable a more rapid and powerful response when that pathogen is subsequently re-encountered. *S. pneumoniae* colonisation of the nasopharynx elicits an adaptive immune response that can help prevent colonisation, pneumonia and invasive disease. Conversely, immune deficiencies that cause defects in the adaptive immune response such as antibody deficiency (73) and T cell deficiencies (74) lead to increased rates of *S. pneumoniae* pneumonia.

> S. pneumoniae colonisation of the nasopharynx elicits an adaptive immune response that can help prevent colonisation, pneumonia and invasive disease.

B lymphocyte and antibody

Prior infection will lead to induction of an antibody response by B lymphocytes to specific bacterial antigens. The high incidence of lung infections in subjects with antibody deficiencies and the efficacy of antibody-inducing vaccines at preventing *S. pneumoniae* pneumonia in children demonstrate a key role for antibody for protection against *S. pneumoniae*. In addition, the turning point described by 19th and early 20th century accounts of pneumonia when patients started to improve potentially reflect the point when the immune system has generated capsular serotype specific antibody. Antibody binding to *S. pneumoniae* improves immunity by activating complement deposition on the bacteria, so promoting complement-mediated phagocytosis, and by direct promotion of phagocytosis through recognition of the antibody by Fcγ receptors expressed by macrophages and neutrophils. In addition, antibody can cause bacterial agglutination which improves removal of *S. pneumoniae* from the respiratory tract by the physical defences (75), and is likely to be how the present vaccine used in children can prevent nasopharyngeal.

S. pneumoniae antigens recognised by naturally ac-

quired (via colonisation or previous infection) or vaccine-induced adaptive immunity include a range of bacterial proteins and the polysaccharide capsule. Polysaccharide antigens such as the *S. pneumoniae* capsule generate a weak adaptive immune response as these antigens are unable to recruit T lymphocytes to assist B cell antibody responses. Although this type of T cell-independent activation generates rapid B cell proliferation and antibody production, it does not generate a memory B cell population that is required for a longer term and recall response on reinfection or revaccination (76). Furthermore, due to immaturity of the immune system, vaccination with capsular polysaccharide alone is ineffective in infants. In contrast, protein antigens stimulate a T-cell dependent B lymphocyte antibody response that generates a memory B cell population and can therefore lead to a recall response to revaccination or infection, and are effective in infants. This effect of protein antigens has been exploited to overcome the limitations of polysaccharide as an immunogen; recent *S. pneumoniae* vaccines conjugate capsular polysaccharide to carrier protein adjuvants to induce a T cell dependent antibody response to the capsular antigen. As a consequence, the conjugated vaccine elicits protective antibody responses to *S. pneumoniae* capsular polysaccharide in infants and has been very effective at preventing *S. pneumoniae* infections, including pneumonia, caused by the serotypes covered by the vaccine (77).

Immunity to *S. pneumoniae* after nasopharyngeal colonisation in mice is dependent on antibody that recognises several *S. pneumoniae* cell wall proteins (78). Antibodies to these protein antigens are also present in human sera and may contribute towards naturally acquired immunity, but it is unclear at present whether antibody responses to the capsule or to a range of protein antigens are the dominant component of protective antibody responses in humans. Several of these protein antigens are at least partially conserved between all *S. pneumoniae* and could form the basis of a novel vaccine, especially if protein antigens are used in combination (79).

T lymphocytes

Cellular adaptive immune responses are driven by T lymphocytes, which fall into 2 broad categories: CD4 expressing helper T cells that secrete cytokines to affect cells in the local milieu, and CD8 expressing cytotoxic T cells that cause apoptosis of host cells infected with intracellular pathogens such as viruses. It is only recently that the importance of cellular responses for adaptive immunity against *S. pneumoniae* infection has been recognised (80).

CD4 Th1 cellular immunity
Th1 cells are a subset of helper T lymphocytes that secrete IFNγ, the effect of which is to 'activate' macrophages to enhance intracellular killing. Their importance during *S. pneumoniae* lung infection is not clear, although the central role of alveolar macro-phages
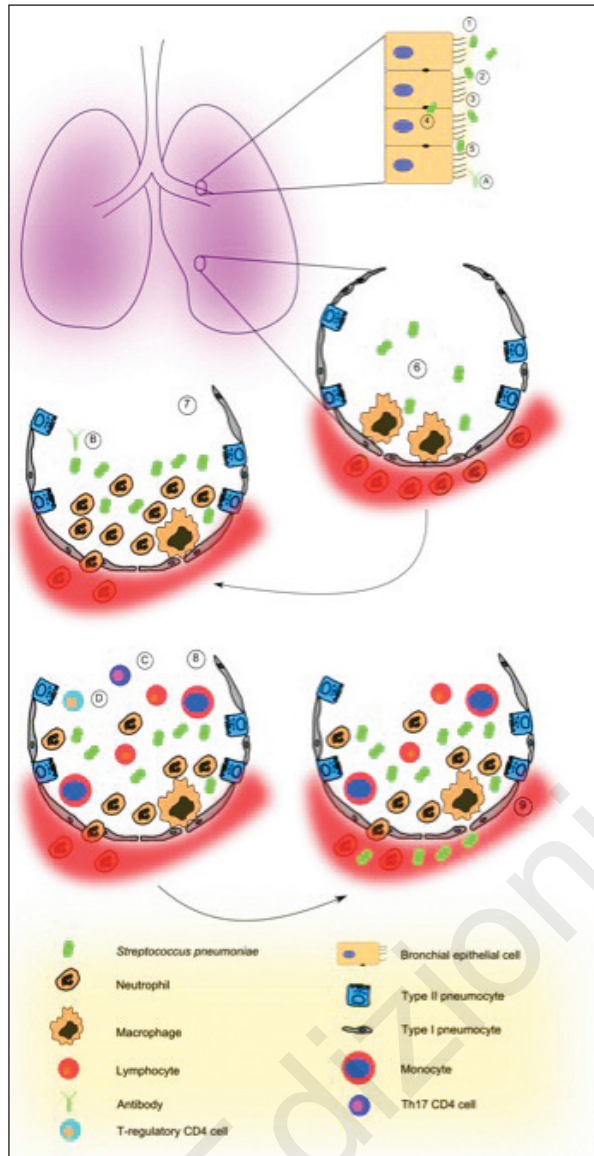
Figure 2 - Schema of confirmed and likely mechanisms of lung immunity to invading *S. pneumoniae*.
Microaspiration of *S. pneumoniae* colonising the nasopharynx allows the bacteria to reach the lungs. There infection is most often controlled by the host's pulmonary innate immune mechanisms. These include the mucosal defences of: (1) Electrostatic repulsion between bacteria and mucous prevents bacteria reaching the mucosal surface; (2) and (3) clearance of bacteria by the mucociliary escalator with the motile cilia moving bacteria imbedded into the mucous layer to the pharynx; (4) tight junctions between the epithelial cells prevent transmigration of the bacteria; and (5) direct killing of the bacteria by lysozyme, lactoferrin, and cationic peptides present in the airway lining fluid. In the airspaces alveolar macrophages (6) are the first immune phagocytes to recognise and kill the pathogen, aided by complement and surfactant. Failure of these initial host defence mechanisms results in replication of the bacteria and the (7) co-ordinated secretion of cytokine and chemokines by epithelial cells and macrophages. This inflammatory response causes rapid recruitment to the alveoli of (8) neutrophils, which kill *S. pneumoniae* by phagocytosis, and (9) exudate containing complement and antibody to improve opsonisation and phagocytosis of the bacteria. Later, monocytes and lymphocytes are recruited to the lungs to assist with bacterial clearing and resolution of inflammation (8). Failure to control bacterial replication in the lung may result in invasive disease whereby the bacteria enter the blood causing septicaemia (9). Adaptive immune responses improve protection against *S. pneumoniae* at multiple levels, usually involving interactions with innate immune mechanisms. These include: (A) antibody mediated agglutination of *S. pneumoniae* improving the rate of bacterial removal by the mucociliary escalator; (B) antibody also improves alveolar macrophage and neutrophil phagocytosis directly through Fc gamma receptors or by increasing complement activity against *S. pneumoniae*; (C) Th17 CD4 cells improve rapid recruitment of neutrophils to the lungs during *S. pneumoniae* pneumonia, and possibly improve epithelial integrity and increase secretion of defensins; (D) T-regulatory CD4 cells help regulate inflammatory responses and thereby may help preserve epithelial barrier integrity and prevent septicaemia.

for control of infection would suggest Th1 adaptive responses should be beneficial. Human monocyte/T cell co-culture with live *S. pneumoniae* does lead to a Th1 cytokine response (81), and the deficiency of the cytokine IL12 (which stimulates Th1 cell proliferation) increases risk of *S. pneumoniae* lung infection (82).

CD4 Th2 cellular immunity
The Th2 subset of CD4 cells secrete IL4, 5 and 13 and improve B cell responses to antigens associated with mucosal disease, however their role in adaptive immune response to *S. pneumoniae* has not been well characterised.

CD4 Th17 cellular immunity
CD4 Th17 cells indirectly improve immunity against mainly extracellular pathogens on mucosal surfaces. After recognition of an invading pathogen, Th17 cells secrete the cytokines IL17, IL17F, and IL22 to improved

mucosal immunity. For example, IL17 induces bronchial epithelium to produce the chemokine CXCL8, resulting in neutrophil recruitment to the site of infection, and β-defensins increasing direct antimicrobial activity. In addition, Th17 responses improve epithelial barrier integrity and increase export of immunoglobulins to mucosal surfaces. Patients with hyper IgE syndrome have defects in Th17 cell differentiation and have increased susceptibility to *S. pneumoniae* pneumonia (83), and experimental human colonisation with *S. pneumoniae* results in detectable Th17 responses in bronchoalveolar fluid and blood (84). These data suggest an important role for CD4 Th17 cells for immunity to *S. pneumoniae*, and this has been confirmed in animal models in which CD4 Th17 responses to colonisation or vaccination can prevent *S. pneumoniae* nasopharyngeal colonisation and pneumonia (85, 86). CD4 Th17 cells recognise *S. pneumoniae* protein antigens rather than the CD4 Th17 cells recognise *S. pneumoniae* protein

antigens rather than capsular polysaccharide, possibly inducing cross erotype protection (87).

CD4 T regulatory cells

Tregulatory cells are largely anti-inflammatory, limiting excessive inflammation by secreting IL10 and reducing IFNγ production. Treg cells are usually associated with poorer outcomes during infection, but recent data suggest they are actually beneficial during *S. pneumoniae* pneumonia . The proposed mechanism is that regulation of the inflammatory response during *S. pneumoniae* pneumonia helps prevent the bacteria from penetrating the epithelial layers to reach the blood and cause septicaemia (88).

CD8 T cells

As CD8 cells are usually associated with responses to intracellular organisms, they are generally thought not to have a role *versus S. pneumoniae*. However, genetic mutations in humans affecting TAP proteins required for CD8 function are associated with an increased incidence of *S. pneumoniae* infection (89), and in one mouse model of serotype 3 *S. pneumoniae* lung infection survival was improved by CD8 positive cells. These data indicate there might be some role for CD8 cells for adaptive immunity to *S. pneumoniae* that requires further characterisation.

Conclusion

The immune mechanisms involved in preventing *S. pneumoniae* lung infections are complex (Figure 2) and incompletely explored. The mucociliary escalator and mucosal antibacterial proteins and peptides inhibit bacterial adherence and survival on the respiratory epithelium. These innate immune mechanisms are supported by alveolar macrophages that attempt to contain infection by phagocytosis whilst avoiding an unnecessarily strong inflammatory response.

If this is unsuccessful, inflammation mediated by the alveolar macrophages and respiratory epithelium recruit neutrophils and monocytes to the lungs thereby increasing phagocytic capacity, and cause an acute phase response and alveolar exudate. Both macrophage and neutrophil phagocytosis is enhanced by the complement molecules and antibody recruited to the site of infection by the inflammatory response. More recent data has shown this schema is relatively simplistic, and that are additional important (and sometimes seemingly contradictory) roles for adaptive cellular immunity, particularly Th17 and Treg cells. A detailed understanding of the interactions between *S. pneumoniae* and host innate and adaptive immune responses is important for understanding the pathophysiology of *S. pneumoniae* pneu-

> **A detailed understanding of the interactions between S. pneumonia and host innate and adaptive responses is important for understanding the pathophysiology of S. pneumoniae pneumonia and the development of novel therapeutic and preventative strategies.**

monia, and may lead to the development of novel therapeutic and preventative strategies.

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