Introduction

This review will focus on two bacterial pathogens, viz Streptococcus pneumoniae, the most common aetiological agent in community-acquired pneumonia, and non-typeable Haemophilus influenzae (NTHi), which, although less common, has taken on greater clinical significance in the post-H. influenzae serotype b (Hib) conjugate vaccine era. Indeed, most invasive H. influenzae diseases in developed countries are now caused by NTHi strains. 1 This represents a potential problem for developing countries because of the inclusion of Hib in childhood vaccination programmes. Both of these bacterial pathogens possess a range of protein and non-protein virulence factors which are essential for both nasopharyngeal colonisation and progression to invasive disease. Notwithstanding current vaccines based on pneumococcal capsular polysaccharides, many of these virulence factors are also candidate antigens for vaccine development. Interestingly, a pneumococcal capsular polysaccharide conjugate vaccine utilising an NTHi carrier protein is a possible future strategy to provide dual immunoprophylaxis.

Identification and prioritisation of candidate antigens on which novel vaccines can be based, or the efficacy of existing vaccines improved, are critically dependent on characterising the strategies utilised by microbial pathogens to evade host defences, and, in particular, the key virulence factors involved. In this review, we have focused on the immune evasion strategies utilised by two important bacterial respiratory pathogens, viz Streptococcus pneumoniae and non-typeable Haemophilus influenzae, with particular emphasis on key virulence factors and their potential to serve as candidate immunogens.

Biofilm

Prior to reviewing pathogen-selective virulence factors, a brief consideration of the role of biofilm in microbial colonisation/persistence is important, as this is a common strategy utilised by the pneumococcus and NTHi to evade host defences. 4 Biofilm is a hydrated, self-generated polymer matrix in which microbial pathogens are effectively insulated against the cellular and humoral defence mechanisms of the host. Biofilm also restricts the penetration of antibiotics, favouring antibiotic resistance. Importantly, both the pneumococcus and NTHi, by the mechanisms described below, adhere to and invade airway epithelium. Biofilm-encased aggregates, in which both pathogens may coexist, either attached to the epithelial surface, or sequestered intracellularly, providing a potential mechanism for bacterial persistence. 4 Concealed in biofilm, in which they communicate by quorum sensing mechanisms, these microbial pathogens can re-emerge at times when host defences are transiently or irreversibly compromised, as may occur during infection with influenza virus/respiratory syncitial virus or HIV-1, respectively.

Key non-protein virulence factors of S. pneumoniae

The antiphagocytic polysaccharide capsule is generally recognised as being the major virulence factor of the pneumococcus, promoting resistance to opsono-phagocytosis and entrapment by mucopolysaccharides present in mucus in the non-immune individual. 5 Although weakly immunogenic, capsular polysaccharides are nevertheless the primary determinants of immune-mediated, type-specific protection against the pneumococcus. They initiate the production of secretory immunoglobulin (Ig)A, an antibody which antagonises adhesion of the pneumococcus to respiratory epithelium, as well as production of both mucosal and circulating IgG antibodies which promote opsono-phagocytosis. The pneumococcus also produces copious amounts of hydrogen peroxide (H$_2$O$_2$) through the action of the enzyme pyruvate oxidase, reaching low millimolar concentrations in bacteriological culture medium. H$_2$O$_2$ is an indiscriminate, cell-permeable oxidant, which is toxic for both eukaryotic and prokaryotic cells. Importantly, H$_2$O$_2$ is cytotoxic for ciliated respiratory epithelium, causing ciliary slowing and epithelial damage, favouring adhesion to, and invasion of, epithelial cells by the pneumococcus. 4 Although potentially suicidal for the pneumococcus, which does not produce catalase, H$_2$O$_2$ also appears to induce biofilm formation, which is likely to favour persistence of the microbial pathogen in the respiratory tract. 4
Table 1: Key non-protein and protein virulence factors of the pneumococcus

<table>
<thead>
<tr>
<th>Factor</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysaccharide capsule</td>
<td>Prevents binding to mucopolysaccharides, antiphagocytic.</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Cytotoxic, impairs mucociliary escalator function.</td>
</tr>
<tr>
<td>Phosphorycholine</td>
<td>Epithelial adhesion.</td>
</tr>
<tr>
<td>Pneumolysin</td>
<td>Cytotoxic, impairs mucociliary escalator function, apoptosis of dendritic cells, complement consumption, proinflammatory.</td>
</tr>
<tr>
<td>Zinc metalloproteinase</td>
<td>Cleaves secretory IgA.</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Potentiates the inhibitory effects of hydrogen peroxide and pneumolysin on mucociliary escalator function.</td>
</tr>
<tr>
<td>PsaA</td>
<td>Epithelial adhesion.</td>
</tr>
<tr>
<td>PspA, PspC</td>
<td>Epithelial adhesion, interference with complement activation.</td>
</tr>
<tr>
<td>PfbB</td>
<td>Epithelial adhesion.</td>
</tr>
</tbody>
</table>

Antagonism of the mucociliary escalator by \( \text{H}_2\text{O}_2 \) acting in concert with the protein virulence factors pneumolysin and hyaluronidase as described below,\(^6,12\) facilitates the attachment of the pneumococcus to airway epithelium via the binding of a third non-protein virulence factor, phosphorycholine on the pneumococcus, to the platelet-activating factor (PAF) receptor on the epithelium.\(^9,19\)

**Key protein virulence factors of *S. pneumoniae***

The pneumococcus also possesses an array of protein virulence factors which contribute to evasion and/or suppression of host defences, some of which are recognised as potential vaccine candidates.\(^4\) These include the choline-binding proteins (Cbps), pneumococcal surface proteins (PspS), neuraminidase, hyaluronidase, a zinc metalloproteinase, divalent metal-binding proteins, the cholesterol-binding, pore-forming toxin pneumolysin, as well as the lipoprotein pneumococcal surface adhesin A (PsaA). During colonisation of the nasopharynx, virulence factors that neutralise both innate and adaptive host defences enable bacterial adhesins to promote attachment of the pneumococcus to airway epithelium. In the case of adaptive host defences, cleavage of secretory IgA by the zinc metalloproteinase favours adhesion of the pneumococcus, while both innate and adaptive host defences are compromised by PspA and PspC (also known as CbpA) which interfere with activation of the alternative pathway of complement activation, as well as by pneumolysin which causes depletion of complement via activation of the classical pathway.\(^4,10\) As alluded to above, pneumolysin, acting in concert with \( \text{H}_2\text{O}_2 \) and hyaluronidase, also interferes with the protective functions of the mucociliary escalator while, in addition, this protein toxin enables the pneumococcus to evade human dendritic cell surveillance by interfering with the maturation of these cells, and by inducing apoptosis.\(^11\)

Notwithstanding the pro-adhesive interactions between pneumococcal phosphorycholine and the PAF receptor on airway epithelium, PspA and PspC also promote attachment via binding to the epithelial polymeric Ig receptor that normally transports secretory IgA, while E-cadherin, the cell-cell junction protein of respiratory epithelium, is a receptor for PsaA.\(^7,12\) Some serotypes possess pilus-like structures that promote epithelial adhesion via interaction with uncharacterised receptors, while a novel pneumococcal protein adhesin has been described recently. This is the 120 kDa plasminogen- and fibronectin-binding protein B (PfbB), which significantly increases the ability of the pneumococcus to adhere to epithelial cells.\(^13\) Unmasking of these various pneumococcal adhesins necessitates a reduction in capsule size, with the accompanying risk of increased vulnerability to phagocytosis. This risk is apparently minimised by production of biofilm, a process in which bacterial neuraminidase,\(^14\) and possibly \( \text{H}_2\text{O}_2 \), participate. These aforementioned virulence mechanisms, which are summarised in Table 1, facilitate translocation of the pneumococcus to the lower airways, while transcytosis of the pathogen across the epithelial barrier via the polymeric Ig receptor enables access to the bloodstream and invasion of the central nervous system.

**Pneumolysin**

Pneumolysin, a key protein virulence factor released upon autolysis of the pneumococcus, merits special mention as this toxin is critically involved in the pathogenesis of severe pneumococcal pneumonia.\(^5,8,15\) In addition to the adverse effects on innate and adaptive host defences mentioned above, the toxin, via its cytotoxic effects on pulmonary endothelial and alveolar epithelial cells, facilitates transmigration of the pneumococcus from the alveoli to the interstitium, and then into the bloodstream. This, in turn, may lead to the development of acute respiratory failure, a serious complication of pneumococcal pneumonia.\(^8,9,15\)

Using an experimental animal model, Wittenrath and colleagues provided revealing insights into the role of pneumolysin in the pathogenesis of acute lung injury. Delivery of pneumolysin into the airways of mice was accompanied by rapid, damaging effects on the alveolar capillary membrane and extracapillary vessels, resulting in vascular leakage and pulmonary oedema, while infusion of the toxin into the pulmonary circulation resulted in pulmonary hypertension.\(^16\) All of these are important features of acute respiratory distress syndrome (ARDS). As mentioned earlier, these direct cytotoxic effects of pneumolysin on airway cellular and structural integrity are likely to be potentiated by \( \text{H}_2\text{O}_2 \) and hyaluronidase, as well as by toxin-mediated, inappropriate activation/exacerbation of inflammatory responses as described below.

**Antipneumococcal host defences**

Although nasopharyngeal colonisation by the pneumococcus is the first stage in invasive disease, it may also protect against severe infection by activating host defences.\(^7\) As mentioned earlier, IgG and secretory IgA antibodies directed against the antiphagocytic, polysaccharide capsule of the pneumococcus are generally considered to be the primary determinants of immune-mediated type-specific protection against the pneumococcus. Antibodies to pneumococcal proteins, particularly CbpA, PsaA, and pneumolysin, also confer protection which, although less efficient, is not serotype restricted.\(^18\) Recently, several additional antipneumococcal host defence mechanisms have been identified which are not dependent on antibody production. These include innate host defences triggered by pneumolysin and lipoteichoic acid (LTA), an
Innate antipneumococcal host defences

Depending on the local density of pneumococci in the airways, pneumolysin, by virtue of its cholesterol-binding pore-forming activity, may either protect against or promote infection. Exposure to small numbers of pneumococci leads to the production of low, sublytic concentrations of pneumolysin which bind to airway epithelium, resulting in subbiotial pore formation and influx of extracellular calcium (Ca\(^{2+}\)), membrane disruption, and osmotic stress.\(^{19-21}\) These events, in turn, lead to the initiation of several intracellular signalling cascades involving \(\alpha\) and \(\beta\) mitogen-activated protein kinases, transforming growth factor-\(\beta\)-activated kinase 1-mitogen-activated protein kinase kinase 3/6-p38 and nuclear factor-kB and nuclear factor of activated T cells, with resultant initiation of synthesis of various proinflammatory chemokines/cytokines, especially the neutrophil chemoattractant interleukin-8 (IL-8), as well as activation of cyclooxygenase 2, by airway epithelium. IL-8, in turn, promotes an influx of neutrophils which contributes to the early control of colonisation.\(^{19}\) This early influx of these cells is also necessary for the degradation of bacteria required for efficient delivery of pneumococcal antigen to nasal-associated lymphoid tissue and induction of mucosal immunity.\(^{22}\) Pneumolysin has also been reported to interact with Toll-like receptor 4 (TLR-4), an event which contributes to both innate and adaptive immunity to the pneumococcus.\(^{23,24}\) In humans, there are currently 10 recognised members of the TLR family, these being the prototype pattern recognition molecules which function as sentinels of the innate immune system. TLRs also link innate and adaptive immunity by promoting the up-regulation of co-stimulatory molecules on antigen-presenting cells, as well as inducing the production of macrophage-activating cytokines by T cells independently of the T cell receptor (TCR) for antigen.\(^{25}\) Interaction of pneumolysin with airway epithelium, as well as with other cells of the innate immune system in the airways, triggers a signalling cascade which leads to the production of IL-1\(\beta\), IL-6, IL-8, and tumour necrosis factor (TNF), all of which cooperate to promote transendothelial migration and chemotaxis of neutrophils. This pneumolysin/TLR-4-mediated inflammatory response may also contribute to the early control of pneumococcal colonisation and promote mucosal immunity.

In addition to the interactions of pneumolysin with TLR-4, LTA and peptidoglycan, both of which are pneumococcal cell wall components, are recognised by TLR-2 on cells of the innate immune system, also resulting in the synthesis of proinflammatory cytokines.\(^{26,28}\) Interestingly, engagement of TLR-4 by pneumolysin has been reported to amplify TLR-2-mediated inflammation by mechanisms which remain to be fully characterised,\(^{24}\) but which may involve increased expression and sensitivity of TLR-2 on immune and inflammatory cells by an interferon-\(\gamma\) (IFN-\(\gamma\))-driven mechanism.\(^{25}\) The early sub-acute, inflammatory response which contributes to the initial control of pneumococcal colonisation of the upper airways therefore appears to require the pore-forming actions of pneumolysin, as well as the interactions of the toxin and LTA/peptidoglycan with TLR-4 and TLR-2, respectively, on cells of the innate immune system, resulting in the recruitment and activation of neutrophils and monocytes/macrophages.

In addition, nucleotide oligomerisation domain (Nod)-like receptors also contribute to innate, antipneumococcal host defences. These are a specialised group of intracellular pattern recognition molecules, which, like TLRs, recognise highly conserved structures on bacterial pathogens, with microbial sensing resulting in activation of cytosolic transcription factors and an inflammatory response.\(^{28}\) In the case of the pneumococcus, bacterial proteoglycans are recognised by epithelial Nod proteins, specifically Nod 2.\(^{29}\)

**Antibody-independent, adaptive, antipneumococcal host defences**

Based on experimental animal and clinico-epidemiological studies, it is now evident that antibody-independent, CD4+ T cell-mediated adaptive immune responses to pneumococcal proteins also contribute to the natural development of immunity to pneumococcal infection, a finding which has significant implications for future vaccine development.\(^{26,31}\) These T cell-mediated immune responses to pneumococcal protein antigens appear to be mediated by CD4+ T cells of both the Th1,\(^{32,33}\) and Th17\(^{32}\) subsets, with involvement of the cytokines IFN-\(\gamma\) and IL-17A, respectively. Although not entirely clear, the mechanism of protection involving Th1 cells may involve enhancement of mannose receptor-mediated phagocytosis and intracellular eradication of the pneumococcus following activation of airway macrophages by IFN-\(\gamma\),\(^{34}\) as well as neutrophil inflitx.\(^{35}\) Importantly, pneumococcal capsular polysaccharides have been shown to bind to the mannose receptor, as well as to other macrophage C-type lectins such as SIGN-R1.\(^{36,37}\) In the case of Th17 cells, production of IL-17A leads to the recruitment of neutrophils and increased pneumococcal killing by these cells.\(^{38}\) Following initial partial control of colonisation by innate nephrophil-mediated mechanisms, recent evidence favours a primary role for Th17 cells in the recruitment of monocytes/macrophages to mucosal surfaces, resulting in clearance of primary bacterial colonisation.\(^{36,38}\) Importantly, TLRs are also expressed on naïve CD4+ T-lymphocytes, as well as on central memory CD4+ T cells and Th1 effector cells, with TLR-1, -5, -7, and -9 messenger RNAs having been detected in these cells.\(^{39-41}\) Interaction of LTA and pneumolysin with TLR-2 and TLR-4, respectively, on T cells is therefore likely to potentiate TCR-mediated immune responses to pneumococcal immunogens. However, exposure of T cells to TLR-2 ligands, such as LTA, also causes T cell activation independently of the TCR, resulting in the production of IL-8 and IFN-\(\gamma\) by naïve T cells and Th1 cells, respectively.\(^{42,43}\) Such a mechanism of TCR-independent activation of CD4+ T cells via TLR-2 (and possibly TLR-4) is also likely to contribute to the control of both pneumococcal colonisation and acute infection.
In summary, pneumococcal colonisation of the airways has several possible outcomes. Firstly, the bacteria may be eradicated by cooperative interactions between innate and adaptive host defence mechanisms. Secondly, and somewhat speculatively, host defences may initially subdue the pneumococcus, which as a survival strategy, protects itself by producing biofilm, entering a quiescent phase of asymptomatic carriage. Thirdly, in highly susceptible individuals, such as the very young, the elderly, or other immunocompromised groups, colonisation may progress rapidly to acute infection.

Adverse effects of inappropriate activation of antipneumococcal host defences

Although the various host defences described above are intended to protect the host against the pneumococcus, hyperactivation of these same mechanisms may occur during acute, poorly-controlled infection. The consequence is excessive production of proinflammatory cytokines and over-exuberant inflammatory responses. In turn, these lead to inflammation-mediated disruption of the epithelial barrier due to the cytotoxic actions of phagocyte-derived reactive oxidant species and proteases, favouring extra-pulmonary dissemination of the pneumococcus. These proinflammatory events are exacerbated by pneumolysin, which not only damages airway epithelium and endothelium directly, but also potentiates inflammatory responses by causing complement activation and hyperactivation of phagocytes. Activation of antipneumococcal host defences may also pose a potential hazard to HIV-infected individuals, for whom the pneumococcus is a major threat. This is because activation via TLR-2 may render naïve and memory CD4+ T cells more susceptible to productive infection by HIV-1 through increased expression of the chemokine receptor, CCR5.

Non-typeable H. influenzae (NTHi)

In contrast to the pneumococcus, NTHi is an unencapsulated, non-toxin-producing bacterial pathogen which is less invasive than either the encapsulated H. influenzae serotype b (Hib) or the pneumococcus. Although most commonly associated with asymptomatic carriage, NTHi is a frequent cause of otitis media, chronic bronchitis and community-acquired pneumonia. Despite the absence of a capsule and a major extracellular toxin, NTHi is nonetheless an astute bacterial pathogen, largely because of its propensity for genetic heterogeneity and antigenic variability, enabling it to evade host defences, while complicating vaccine development. In the post-Hib vaccination era, there is also increasing concern in relation to the rising prevalence of invasive disease due to both NTHi and non-Hib encapsulated strains.1

Non-protein and protein virulence factors of NTHi

Like the pneumococcus, NTHi possesses a range of virulence factors which promote colonisation of the nasopharynx and evasion of host defences. In addition to biofilm and an IgA1 protease, these include the outer membrane lipopolysaccharide (LPS) and an array of protein adhesins and inhibitors of innate host defences.

Lipopolysaccharide

Lipopolysaccharide (LPS) is an essential and characteristic outer membrane component of H. influenzae, also known as lipooligosaccharide (LOS) because it lacks O-specific polysaccharide chains. The LPS of H. influenzae consists of a conserved, glucose-substituted, triheptosyl inner core moiety linked to lipid A, which provides the template for attachment of variable length oligosaccharide and non-carbohydrate (phosphate substituents, O-acetyl groups) ester-linked moieties. Importantly, LPS shows considerable intra-chain and inter-chain heterogeneity of glycoform structure due to the extensive LPS gene repertoire and variable expression profiles, with LPS phase variation being critical for evasion of host defences and persistence. With respect to colonisation, a majority of NTHi strains express phosphocholine on the LPS outer core through the activity of the enzyme, diphosphonucleoside choline transferase. The addition of phosphocholine apparently enables LPS to function as an adhesin as described below. Activation of antipneumococcal host defences may also pose a potential hazard to HIV-infected individuals, for whom the pneumococcus is a major threat. This is because activation via TLR-2 may render naïve and memory CD4+ T cells more susceptible to productive infection by HIV-1 through increased expression of the chemokine receptor, CCR5.

Protein virulence factors of NTHi

Following entry of NTHi into the upper respiratory tract, the bacteria must overcome the expulsive actions of the mucociliary escalator. This appears to be achieved by the outer membrane proteins (OMPs), P2 and P5, and possibly other proteins, which promote bacterial binding to mucus, while protein D, a highly conserved 42 kDa surface lipoprotein with glycosylphosphatidylinositol phosphodiesterase activity found in all strains of H. influenzae, including NTHi, possibly acting in concert with LPS, has been shown to impair mucociliary function. Subsequently, several adhesins, in addition to phosphocholine-expressing LPS mentioned above, promote direct adherence to respiratory epithelium. In approximately 80% of NTHi isolates, the major adhesins are the related proteins, HMW1 and HMW2. These are high molecular weight, non-pili adhesins with broad adhesive capacity, which enable NTHi to adhere to a variety of epithelial cell types. HMW1 appears to interact with a poorly characterised sialylated glycoprotein on epithelial cells, while the receptor for HMW2 is unknown. Other protein adhesins which promote colonisation by NTHi include: i) pili in some NTHi which bind to components of the extracellular matrix; ii) OMPs of the autotransporter family such as Hap, which also binds to extracellular matrix components, and Hia which interacts with an unidentified epithelial receptor; iii) the 16 kDa vitronectin-binding protein, protein E (PE); and iv) P5 which binds to epithelial carcinoembryonic antigen-related cell adhesion molecules.
In addition to promoting binding to the extracellular matrix, PE also captures vitronectin on the surface of NTHi which protects the bacterial pathogen against the lytic activity of the membrane attack complex of the complement system. Like the pneumococcus, NTHi also protects itself against attack by the alternative and classical pathways of complement activation by binding factor H and C4BP, respectively.

The aforementioned virulence factors of NTHi are summarised in Table 2.

**Table 2: Key non-protein and protein virulence factors of NTHi**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide</td>
<td>Epithelial adhesin, interference with complement activation, impairs mucociliary escalator function.</td>
</tr>
<tr>
<td>P2, P5</td>
<td>Promotes binding to mucus, adhesion to epithelium (P5).</td>
</tr>
<tr>
<td>Protein D</td>
<td>Impairs mucociliary escalator functions.</td>
</tr>
<tr>
<td>HMW1, HMW2</td>
<td>Epithelial adhesin.</td>
</tr>
<tr>
<td>Hia</td>
<td>Epithelial adhesin.</td>
</tr>
<tr>
<td>Pii, Hap, Protein E</td>
<td>Promotes binding to the extracellular matrix, protects against the lytic action of complement (PE).</td>
</tr>
</tbody>
</table>

**Advances in immunisation against the pneumococcus and future strategies**

Although considerable progress has been made in the field of immunisation against pneumococcal diseases, particularly the successful development of conjugate vaccines which effectively reduce colonisation and disease caused by vaccine-type strains, serotype restriction remains a major limitation of current vaccines. Moreover, recent studies have identified the existence of antibody-independent, cell-mediated immune responses triggered by pneumococcal protein antigens, which contribute to clearance of colonisation and possibly prevention of mucosal disease, clearly highlighting an additional limitation of current vaccines, none of which contains pneumococcal protein antigens.

Accordingly, future vaccines will focus on the utilisation of highly-conserved, broadly serotype-unrestricted, recombinant, surface and sub-surface pneumococcal protein antigens. Ideally, these should evoke the production of both neutralising antibodies and cell-mediated immune responses. Prominent among the protein antigen vaccine candidates are PsaA, PspA, PspC/CbpA, and immunogenic pneumolysoid, a modified pneumolysin devoid of pore-forming activity. There are several possible types of protein-based vaccines. These are: i) those which contain a single protein; ii) those which contain a cocktail of different proteins; iii) a conjugate vaccine in which a single protein is used as a carrier for pneumococcal capsular polysaccharides; and iv) a conjugate vaccine in which a cocktail of proteins is used as a carrier system for pneumococcal polysaccharides.

**NTHi-targeted vaccines**

Heterogeneity of several major surface antigens, genetic heterogeneity of strains, and identification of conserved immunogens which evoke broadly protective immune responses, have been the major problems confronting NTHi vaccine development. Protein D has been identified as a promising vaccine candidate antigen in animal models of experimental infection. In a clinical trial involving children in which PD was used as an immunogenic carrier protein for pneumococcal polysaccharides, a modified pneumolysin devoid of pore-forming activity. There are several possible types of protein-based vaccines. These are: i) those which contain a single protein; ii) those which contain a cocktail of different proteins; iii) a conjugate vaccine in which a single protein is used as a carrier for pneumococcal capsular polysaccharides; and iv) a conjugate vaccine in which a cocktail of proteins is used as a carrier system for pneumococcal polysaccharides.

**Host defences operative against NTHi**

As with the pneumococcus, entry of NTHi into the nasopharynx has several possible outcomes: i) eradication in innate/adaptive host defences; ii) a carrier state in which the bacteria and host co-exist in relative harmony; iii) a state of persistence in which the bacteria are encased in biofilm either extracellularly, or intracellularly following invasion of epithelial cells, enabling evasion of host defences (this could also be considered as being a chronic infection); and iv) acute infection. In the case of the carrier and persistence states, the bacterial pathogen, seemingly subdued, may strike when host defences are transiently compromised, as may occur during infection with influenza or other respiratory viruses.

In addition, specific mucosal and circulating IgA and IgG antibodies to surface proteins of NTHi, innate host defences including the mucociliary escalator, antimicrobial proteins in epithelial lining fluid, TLR/Nod-activated inflammatory responses, and activation of the alternative complement pathway are all involved in controlling colonisation. In the case of TLR-mediated inflammatory responses, OMP-2 and OMP-6 bind to TLR-2, while LPS interacts with TLR-4, resulting in transcription factor activation and initiation of an inflammatory response. Recognition of LPS is mediated by its receptor, CD14, on cells of the myelomonocytic lineage, with the CD14/LPS complex being the apparent ligand for TLR-4. An additional protein, MD-2, is associated with TLR-4 and is necessary for the correct positioning of TLR-4 and its interaction with LPS. Intra-epithelial sensing of NTHi proteoglycan by Nod 1 also initiates the production of proinflammatory cytokines. In addition to the aforementioned innate host defences, LPS is also a potent activator of the alternative pathway of complement activation, resulting in the production of complement-derived mediators of inflammation, opsonophagocytosis, and antimicrobial activity.