Mini-Review

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Innate immunity and the pneumococcus

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The innate immune system provides a non-specific first line of defence against microbes and is crucial both in the development and effector stages of subsequent adaptive immune responses. Consistent with its importance, study of the innate immune system is a broad and fast-moving field. Here we provide an overview of the recent key advances made in this area with relation to the important pathogen *Streptococcus pneumoniae* (the pneumococcus).

Introduction

Innate immunity covers a diverse array of host defences, including mucociliary clearance, complement, neutrophils and macrophages. It acts as a non-specific defence able to recognize and respond rapidly against a broad range of microbes. Unlike adaptive immunity, which involves the clonal expansion of T and B cells specific to the pathogen, innate recognition is achieved through a limited set of germline-encoded receptors and does not possess immunological memory. The two systems do not however operate in isolation, as the innate immune system plays crucial roles in the initiation, development and effector stages of adaptive immunity. Recent advances in innate immune recognition and response to the pneumococcus (*Streptococcus pneumoniae*) are discussed here. Better understanding of these interactions may ultimately aid therapy through the promotion of protective responses and the inhibition of deleterious ones.

Complement and the pneumococcus

The complement system comprises over 30 serum and membrane proteins which, when activated, form a cascade of reactions contributing to the elimination of invading micro-organisms. The binding and activation of complement components to the surface of a microbe leads to opsonophagocytosis and the induction of inflammation. For some organisms, but not the pneumococcus, complement can destroy the microbe directly through lysis by the membrane attack complex. Complement contributes to and links both innate and adaptive immunity. Three pathways of activation exist (reviewed by Walport, 2001); in brief, these are:

- 1) The classical pathway activated by antibody–antigen complexes; for example, antibody binding to the microbial surface. Non-antibody-dependent activation can also occur, such as the binding of acute-phase proteins to the micro-organism.
- 2) The lectin pathway, which is triggered by mannose-binding lectin recognition of carbohydrate on the microbial surface.

- 3) The alternative pathway, which is continuously activated at low levels, but only amplifies on foreign surfaces.

The crucial contribution of complement to innate and adaptive responses to pneumococcal infection is long established, both in animal models and in humans with genetic complement deficiencies (for recent overview see Hostetter, 2004). However, only recently, through the use of a panel of gene knock-out mice lacking various complement components, has the relative importance of individual activation pathways been assessed in innate immunity to pneumococcal infection (Brown *et al.*, 2002). The classical pathway of complement activation has been found to be the dominant complement pathway for innate immunity to the pneumococcus in mice (Brown *et al.*, 2002), the specific loss of which results in significantly increased disease severity. Natural IgM antibodies, possibly to C polysaccharide (teichoic acid), contribute to this activation of the classical pathway, as shown by the use of $\mu_s^{-/-}$ knock-out mice which lack such antibodies (Brown *et al.*, 2002). However, the activation of the classical pathway during this innate immune response is only partially dependent on natural antibodies, and other activation pathways also contribute. These are proposed to include acute-phase proteins, such as C-reactive protein, or direct binding of complement component C1q to the pneumococcal surface (Brown *et al.*, 2002). The alternative pathway also contributes to protective innate responses, but to a lesser degree than seen for the classical pathway, while the role for the lectin pathway appears negligible. In line with this latter finding, genetic mannose-binding lectin deficiency is associated with only a small (but significant) increased susceptibility to pneumococcal disease in humans (Roy *et al.*, 2002). Regardless of the activation pathway, the deposition and activation of complement component C3 on the bacterial surface is a key step in the complement cascade leading to elimination of the microbe. In accordance with this crucial role of complement in innate immunity, the pneumococcus has evolved several mechanisms to resist its effects. The capsule is a key factor in this resistance, not only acting to limit access to cell-bound complement but also reducing the amount of complement

deposited (Abeyta *et al.*, 2003). The pneumococcal surface protein PhpA [also called PhtB and BVH-11 (Hamel *et al.*, 2004)] has been found to possess C3-degrading activity (Angel *et al.*, 1994; Zhang *et al.*, 2001), and so PhpA may contribute to the prevention of complement-mediated clearance. In addition to its cytolytic activity, the toxin pneumolysin, a major pneumococcal virulence factor, has multiple other biological activities (for review see Mitchell, 2004). Recently, pneumolysin has been shown to confer protection from complement-mediated clearance (Yuste *et al.*, 2005). Deletion of pneumolysin causes an increase in C3 deposition on pneumococcal cells *in vitro*. Showing that this activity of pneumolysin contributes to virulence, absence of complement in gene knock-out mice reduces the importance of pneumolysin to pneumococcal virulence. Furthermore, this effect of pneumolysin *in vitro* and *in vivo* is specific to the classical and not the alternative activation pathway (Yuste *et al.*, 2005). The mechanisms by which pneumolysin achieves this effect are as yet unconfirmed. However, this specific evasion of the classical pathway is in agreement with the ability of pneumolysin to activate this pathway in the absence of specific toxin antibodies (Mitchell *et al.*, 1991; Paton *et al.*, 1984). Thus, complement evasion in this instance may ironically be the result of complement activation rather than inhibition. Released pneumolysin may result in complement activation away from the bacterial cell, thus protecting it and also consuming the available complement components. In addition, increased complement activation may contribute to host-tissue damage, thereby promoting bacterial pathogenesis. Furthermore, the surface

proteins PspA and PspC also contribute to complement resistance, but these proteins are covered in a companion review in this issue (Bergmann & Hammerschmidt, 2006).

Pattern recognition receptors

Key components of the innate immune system are so-called pathogen recognition receptors (PRRs). These can be located on the host cell surface, intracellularly or be secreted and act to initiate the recognition of and response to microbes and in some cases host products (Janeway & Medzhitov, 2002). The microbial components recognized by PRRs are referred to as pathogen-associated molecular patterns (PAMPs), so-called because they are typically invariant structures found among many microbes but absent in eukaryotes. The use of the term pathogen is potentially misleading, as many PAMPs are conserved among both pathogens and non-pathogens. Examples include lipopolysaccharide (LPS) and peptidoglycan from bacteria, double-stranded RNA from certain viruses and mannan from fungi. C-reactive protein (CRP) is a well-known example of a PRR involved in the response to the pneumococcus. This soluble protein binds phosphorylcholine in the pneumococcal cell wall, inducing complement activation and leading to bacterial clearance (Mold *et al.*, 2002; Szalai *et al.*, 1997). Additional PRRs important in pneumococcal infection have recently been described, including members of the toll-like receptor (TLR) family, discussed below. The key PRRs discussed in this review are shown in Fig. 1.

Fig. 1. Key pathogen recognition receptors involved in the recognition and initiation of the immune response towards the pneumococcus. Abbreviations: LBP, LPS binding protein; LTA, lipoteichoic acid; TLR, toll-like receptor.

Toll-like receptors

The TLR family of PRRs has received much attention due to their importance in the response to a wide range of microbes (for reviews see Hopkins & Sriskandan, 2005; Takeda *et al.*, 2003). Their major function as PRRs is to recognize microbes and initiate an inflammatory response leading to eradication of infection. At least ten TLRs have been described in humans and mice, and several have been implicated in pneumococcal infection in animal models. Furthermore, descriptions of genetic defects in TLR signalling associated with increased susceptibility to pneumococcal disease show that these receptors have relevance to human infection (Currie *et al.*, 2004; Picard *et al.*, 2003).

TLR2. TLR2 recognizes both pneumococcal lipoteichoic acid (LTA) and cell wall peptidoglycan (Han *et al.*, 2003; Schroder *et al.*, 2003; Weber *et al.*, 2003; Yoshimura *et al.*, 1999). Interestingly, despite numerous studies supporting the recognition of bacterial peptidoglycan via TLR2, this interaction has recently been challenged (Travassos *et al.*, 2004). Echchannaoui *et al.* (2002) show that TLR2^{-/-} mice display increased disease severity and decreased survival times compared with wild-type mice in a pneumococcal meningitis model. This greater susceptibility correlates with heightened bacterial levels in the brain but appears to be independent of systemic disease, as both strains show similar bacterial levels in the blood. A transient delay in leukocyte recruitment into the brain potentially contributes to increased bacterial outgrowth and disease progression in the knock-out mice. Despite this slower early leukocyte recruitment, knock-out mice show enhanced inflammation in the brain later in infection. This is perhaps due to the increased bacterial levels providing a greater inflammatory stimulus, which may in turn contribute to increased disease through immunopathology.

In agreement with these data, Koedel *et al.* (2003) have also found in their meningitis model that TLR2^{-/-} mice have enhanced disease and increased bacterial levels in the brain.

The role of TLR2 has also been investigated in experimental pneumococcal pneumonia (Knapp *et al.*, 2004). Comparison between wild-type and TLR2^{-/-} mice following intranasal infection reveals only a modest contribution for this receptor in the host response. Although TLR2 knock-out mice show reduced pulmonary inflammation, no difference is seen with regard to bacterial clearance and morbidity compared with their wild-type counterparts. On the basis of these data, TLR2 does not appear to play a key role in host resistance to pneumococcal pneumonia. Interestingly, the stimulation of isolated alveolar macrophages *in vitro* to produce TNF- α in response to heat-killed pneumococci is entirely dependent on TLR2 (Knapp *et al.*, 2004). However, immunohistochemical staining of infected lungs from TLR2^{-/-} mice shows that these cells are producing TNF- α at a level comparable to that of wild-type mice. Presumably in the setting of the intact animal, other host innate immune factors, such as other PRRs and complement, mask the loss

of TLR2, rendering its influence minimal in pneumococcal pneumonia. It is clear that the complexity of the immune response, with numerous interacting factors, makes the use of intact animal models an essential tool.

Following intraperitoneal infection, TLR2 knock-out mice have slightly reduced survival times compared with wild-type (Khan *et al.*, 2005). Thus, TLR2 has a protective role in this model of systemic infection, although as with pneumonia, the defect is arguably minor.

Furthermore, in a model of nasopharyngeal colonization, TLR2 knock-out mice have impaired clearance of pneumococci (van Rossum *et al.*, 2005), showing that TLR2 is of relevance not only to disease states but also carriage.

TLR4. Through the recognition of LPS, TLR4 is a key component of the innate response to Gram-negative infections. A role for this receptor has also been extended to the pneumococcus with the finding that the *in vitro* pro-inflammatory affect of pneumolysin on macrophages is TLR4 dependent (Malley *et al.*, 2003). Subsequently, pneumolysin has been shown to directly interact with TLR4 (Srivastava *et al.*, 2005). This inflammatory activity is not dependent on the pore-forming or complement-activating activities of pneumolysin because the PdT pneumolysin mutant, which lacks these properties, was also active. The significance of this interaction during colonization has been studied by comparing wild-type and TLR4-deficient mice in a nasopharyngeal carriage model (Malley *et al.*, 2003). In the absence of functional TLR4, mice are more heavily colonized and much more likely to develop invasive disease. Thus, through its recognition of pneumolysin, TLR4 acts in the nasopharynx to limit pneumococcal proliferation. While the inflammatory response to pneumolysin may contribute to this protection, it has also been shown that pneumolysin-TLR4 signalling can induce host-cell apoptosis *in vitro* and *in vivo* (Srivastava *et al.*, 2005). This also appears to be a protective host response, as the inhibition of apoptosis renders mice more susceptible to death following pneumococcal infection (Srivastava *et al.*, 2005). Interestingly, a similar model of colonization using the same mouse strains has found no difference in the clearance of pneumococci between wild-type and TLR4-deficient mice (van Rossum *et al.*, 2005). The use of different bacterial strains in these studies may explain this apparent conflict, but this remains to be tested. In pneumococcal pneumonia, TLR4 also plays a protective role (Branger *et al.*, 2004b). In this experimental model, the absence of functional TLR4 renders mice more susceptible to morbidity with increased bacterial counts in the lungs. The effects, however, are modest, with the effect on death rate only apparent at low doses and with no significant impact on pulmonary inflammation. Furthermore, the significance of TLR4 in pneumococcal infections appears restricted to the airway surfaces, as earlier work has found that the absence of TLR4 makes no difference

to survival rates and blood bacterial counts after intravenous infection of mice (Benton *et al.*, 1997).

TLR9. Bacterial DNA has inflammatory properties resulting from the presence of unmethylated cytosine-phosphate-guanosine (CpG) motifs (for review see Krieg, 2002). Unmethylated CpG motifs are therefore an additional PAMP and have been shown to be recognized by and to confer their activity via TLR9 (Bauer *et al.*, 2001). The autolytic nature of the pneumococcus with resultant DNA release (Moscato & Claverly, 2004) may make the interaction between bacterial DNA and TLR9 important during pneumococcal infection. This supposition is untested, and it would be of interest to investigate the response to pneumococcal infection in TLR9-deficient mice. Interestingly, the transfection of HEK293 cells with TLR9 fails to confer responsiveness to the pneumococcus, whereas TLR2 and TLR 4 do (Koedel *et al.*, 2003). Therefore the role of TLR9 in pneumococcal infections remains to be confirmed.

TLR1 and TLR6. The recognition of pneumococcal peptidoglycan probably involves interaction between TLR2 and TLR6, as shown for *Staphylococcus aureus* peptidoglycan (Ozinsky *et al.*, 2000). Indeed, confirming a role for this receptor in pneumococcal recognition, the expression of a double negative TLR6 mutant inhibits TNF- α production in response to stimulation by the pneumococcus in a macrophage cell line (Ozinsky *et al.*, 2000). A role exists for TLR1 in the recognition of pneumococcal LTA, whereby monoclonal antibodies against this receptor inhibit LTA-induced TNF- α production from human peripheral blood mononuclear cells (Han *et al.*, 2003). The importance of these interactions between the pneumococcus and TLR1 and TLR6 has not yet been assessed in an infection model.

TLR signalling. Myeloid differentiation factor 88 (MyD88) is a key adaptor molecule in the signalling cascade activated by engagement of TLRs or interleukin-1 (IL-1) family receptors (Yamamoto *et al.*, 2004). In agreement with a role for TLRs in innate protection against pneumococcal infection, MyD88^{-/-} mice show enhanced susceptibility to *S. pneumoniae* in different infection models (Albiger *et al.*, 2005; Khan *et al.*, 2005; Koedel *et al.*, 2004). Providing relevance to human infection, deficiency in IL-1 receptor-associated kinase 4 (IRAK4), also a mediator in the TLR/IL-1 receptor signalling pathway, results in increased susceptibility to pneumococcal disease (Picard *et al.*, 2003), as does a distinct, but as yet undefined, mutation in this signalling pathway (Currie *et al.*, 2004).

LPS binding protein (LBP)

In addition to TLR2, the pneumococcal cell wall (PCW) is recognized by the soluble acute-phase protein LBP (Weber *et al.*, 2003). This protein has previously been found to bind LPS and enhance its inflammatory activity. It has now also been found to bind purified PCW and whole pneumococci,

and the addition of LBP accentuates the inflammatory activity of PCW *in vitro* (Weber *et al.*, 2003). This has been extended to a mouse meningitis model, in which LBP gene knock-out mice show significantly reduced meningeal inflammation following challenge with purified cell wall or live pneumococci. Clinical outcome was not assessed, but in this setting it might be predicted that the absence of LBP would be beneficial. Recognition of PCW by LBP appears independent of cell wall phosphorylcholine and teichoic acid, with the glycan backbone seemingly a crucial structure for this interaction (Weber *et al.*, 2003). Significance to human infections is shown by enhanced LBP levels in the cerebrospinal fluid (CSF) of pneumococcal patients compared with controls. Furthermore, PCW coprecipitated with LBP in the CSF from a patient, showing that this interaction occurs in human infection (Weber *et al.*, 2003). LBP also contributes to the response to pneumococcal LTA (Schroder *et al.*, 2003). However, LBP gene knock-out mice do not have altered susceptibility or responses to pneumococcal pneumonia (Branger *et al.*, 2004a). Given that LBP acts to facilitate recognition of TLR2 ligands, this phenotype is compatible with the modest effect seen with TLR2 knock-out mice in similar infections (Knapp *et al.*, 2004). Interestingly, LBP levels in lavage fluid increase sevenfold during experimental pneumococcal infection (Branger *et al.*, 2004a). This is a much greater increase than that seen with parallel *Klebsiella pneumoniae* pneumonia (Branger *et al.*, 2004a). However, endogenous LBP contributes significantly to protection against *K. pneumoniae* pneumonia, which is not the case for pneumococcal pneumonia (Branger *et al.*, 2004a). Clearly, this highlights that functional importance cannot easily be predicted from expression levels alone.

CD14

Membrane-bound and soluble CD14 acts as a co-receptor to enhance the response to LPS (Abeyta *et al.*, 2003; Pugin *et al.*, 1993, 1994). CD14 also contributes to the recognition of the pneumococcus *in vitro* (Cauwels *et al.*, 1997; Han *et al.*, 2003; Schroder *et al.*, 2003; Yoshimura *et al.*, 1999). A role in infection has also been confirmed by the recent finding that CD14 gene knock-out mice show exacerbated pneumococcal meningitis (Echchannaoui *et al.*, 2005).

Nod proteins and the pneumococcus

The cytosolic proteins Nod1 and Nod2 are additional PRRs acting within host cells to recognize and respond to microbial products (Philpott & Girardin, 2004). In addition to TLRs, a role for Nod proteins in the recognition and response to pneumococci has also been shown (Opitz *et al.*, 2004). Transfection with Nod2, but not Nod1, confers responsiveness to cells following pneumococcal exposure, as judged by NF- κ B activation (Opitz *et al.*, 2004). In line with the role of Nod proteins in the response to intracellular material, the recognition of pneumococcus by Nod2 is dependent on internalization of the bacteria. Nod2 has previously been shown to be responsive to a muramyl dipeptide conserved in multiple peptidoglycans, and this is

the suggested mechanism for its recognition of the pneumococcus (Opitz *et al.*, 2004). The availability of Nod1 and Nod2 knockout mice will allow a fuller appreciation of these genes in the host response to the pneumococcus (Chamaillard *et al.*, 2003; Girardin *et al.*, 2003; Pauleau & Murray, 2003).

Recognition of capsule

Recognition of the pneumococcal polysaccharide capsule by PRRs has received much attention of late. Kang and co-workers showed that the C-type lectin SIGN-R1 expressed by macrophages, particularly in the marginal zone of the mouse spleen, binds capsular polysaccharide from several different serotypes, as well as whole pneumococcal cells (Kang *et al.*, 2004). Lanoue *et al.* (2004) have demonstrated a functional significance of this interaction with the generation of SIGN-R1 gene knock-out mice. Following intraperitoneal infection with either serotype 2 or serotype 14 pneumococci, these mice display increased susceptibility to infection compared with their wild-type counterparts. Absence of SIGN-R1 causes a decrease in survival rate, shorter time to death, increased sickness scores and increased bacterial levels in the blood. A defect in the ability of macrophages in the peritoneum and spleen to bind and phagocytose the pneumococcus is likely to be a major contributor to the increased susceptibility of the knock-out mice (Lanoue *et al.*, 2004).

The role of SIGN-R1 in pneumococcal pneumonia has also been investigated with the use of SIGN-R1-deficient mice (Koppel *et al.*, 2005). When infected intranasally with a serotype 3 strain, the SIGN-R1 knock-out mice show increased bacterial levels in the lungs compared with wild-type. This is accompanied by a higher incidence of bacteraemia, and increased bacterial counts in the blood and spleen. Interestingly, alveolar macrophages do not express SIGN-R1, and expression is not induced following pneumococcal infection (Koppel *et al.*, 2005). This suggests that the protective role of SIGN-R1 in pneumonia does not occur within the lungs themselves. One potential mechanism for the increased bacterial growth in the absence of SIGN-R1 is found to be reduced levels of anti-phosphorylcholine IgM. In addition, systemic disease in this model is probably exacerbated by defective phagocytosis by macrophages in the peritoneum and spleen, as described by Lanoue *et al.* (2004). Therefore, in both pulmonary and systemic infections, SIGN-R1 is instrumental in host resistance to the pneumococcus.

Another macrophage receptor, MARCO, has also recently been identified as important in pneumococcal infection (Arredouani *et al.*, 2004). Genetic deletion of this scavenger receptor renders mice more susceptible to pneumococcal pneumonia, with impaired bacterial clearance from the lungs and increased morbidity (Arredouani *et al.*, 2004). Isolated alveolar macrophages from the knock-out mice are impaired in their ability to bind and phagocytose the pneumococcus *in vitro*, and this is likely a key factor in the increased susceptibility to infection. Interestingly, reduced

phagocytosis is not due solely to reduced bacterial binding, and so a role for MARCO appears to exist not only in binding but in subsequent bacterial uptake. The pneumococcal ligands recognized by MARCO have not yet been identified.

Previously, pneumococcal capsular polysaccharide has been shown to activate macrophages (Um *et al.*, 2000), an activity partially dependent on CD14; whether or not this activity also involves SIGN-R1 and MARCO remains to be determined.

Surfactant proteins

Pulmonary surfactant is a mixture of lipids and proteins that act to prevent alveoli from collapsing during expiration. In addition, the surfactant proteins (SPs) SP-A and SP-D play a role in innate immunity against a variety of pathogens, acting by binding microbes and promoting their phagocytosis or by modulating immune-cell function (Crouch & Wright, 2001; Whitsett, 2005). In the case of pneumococcal infection, SP-D knock-out mice show enhanced susceptibility to intranasal infection (Jounblat *et al.*, 2005). SP-A has recently been shown to promote phagocytosis of *S. pneumoniae* by rat and mouse alveolar macrophages *in vitro* (Kuronuma *et al.*, 2004).

CD4⁺ T cells in innate immunity to the pneumococcus

The function of CD4⁺ T cells in adaptive immunity is well established. Interestingly, they appear also to contribute to early resistance to pneumococcal infection independently of their role in adaptive antigen-specific responses (Kadioglu *et al.*, 2004). Intranasal infection of MHCII knock-out mice, which display a significant decrease in CD4⁺ T cell levels, has revealed a key role for these cells in the early response to pneumococcal pneumonia. These mice display increased susceptibility to infection, as evidenced by increased bacterial counts in the lung and blood compared with their wild-type counterparts. Indeed, the increased susceptibility is so great that it results in 100 % mortality in the knock-out mice by 3 days post-infection, whereas all wild-type mice survive the challenge. In accordance with previous data showing T cell migration to infected lung tissue in pneumococcal pneumonia (Jounblat *et al.*, 2003; Kadioglu *et al.*, 2000), purified CD4⁺ T cells migrate to the pneumococcus *in vitro*. This migration is associated with T cell activation, and interestingly occurs only in response to *in vivo* and not to *in vitro* grown bacteria. Pneumolysin plays a significant part in this migration, as pneumolysin-deficient pneumococci stimulate significantly less cell migration. How pneumolysin stimulates these T cells is unclear, but the recent description of TLR4 expression by T cells may be of relevance (Komai-Koma *et al.*, 2004). Recently, Malley *et al.* (2005) have demonstrated a crucial role for CD4⁺ T cells in antibody-independent acquired immunity to pneumococcal colonization. How CD4⁺ T cell migration and activation in response to the pneumococcus, as described in pneumonia,

relates to this acquired immunity to colonization is as yet unclear.

Innate immunity and interaction between the pneumococcus and other microbes

For ease of study, most work on the interaction of the pneumococcus with the innate immune system has employed pure cultures. However, the mucosa of the upper respiratory tract is colonized by a diverse array of microbial species. Indeed, analysis of DNA from human airway surface fluid suggests the presence of more than 500 bacterial species (Paster *et al.*, 2001). Concurrent stimulation of the innate immune system by multiple species appears to have effects distinct from those of single-species interactions (Neish *et al.*, 2000; Tong *et al.*, 2003), and this has recently been shown to have relevance to the pneumococcus (Ratner *et al.*, 2005). Co-stimulation of human respiratory epithelia cells *in vitro* by *S. pneumoniae* and *Haemophilus influenzae*, also an inhabitant of the upper respiratory tract, results in synergistic production of IL-8 (Ratner *et al.*, 2005). This has been extended to a mouse-colonization model, with a synergistic effect on MIP-2 production and inflammatory influx into the upper airways. This synergy is independent of TLR2 and TLR4, but involves NF- κ B translocation to the nucleus and phosphorylation of p38 MAPK. With regard to the microbial products involved, pneumolysin can substitute for the pneumococcus, but the PdB pneumolysin toxoid, lacking cytolytic activity, is inactive. It is therefore speculated that the pore-forming activity of pneumolysin leads to enhanced delivery of microbial products, such as the soluble inflammatory protein SCF from *H. influenzae*, into the host cell, where recognition by Nod1 and Nod2 would result in increased stimulation (Ratner *et al.*, 2005). This pro-inflammatory activity of pneumolysin is therefore distinct from its effects on macrophages that are mediated through TLR4 and are independent of pore-forming activity.

The significance of pneumococcal-*H. influenzae* interactions has recently been examined in a co-colonization mouse model (Lysenko *et al.*, 2005). In contrast to what might be expected based on *in vitro* studies with these bacteria (Pericone *et al.*, 2000; Shakhnovich *et al.*, 2002), co-colonization *in vivo* results in rapid clearance of the pneumococcus. This effect is dependent on the innate immune system in the form of neutrophils and complement, with the depletion of either abolishing the competitive effect. Activation of peritoneal neutrophils with heat-killed *H. influenzae* causes an increase in their ability to kill the pneumococcus, but has no effect on their ability to kill *H. influenzae* (Lysenko *et al.*, 2005). The basis of this activity is not yet clear. Thus, interactions with the innate immune system can have a significant effect during competition between the pneumococcus and other microbes in the nasopharynx.

Another important microbial interaction is that of the pneumococcus and the influenza A virus. Subsequent to influenza A outbreaks, secondary pneumococcal infection

is an important cause of morbidity and mortality. This heightened susceptibility to pneumococcal disease can be reproduced in animal models, allowing investigation of the mechanisms involved. While viral neuraminidase contributes to this phenomenon by exposing pneumococcal receptors (McCullers & Bartmess, 2003; Tong *et al.*, 2001), alterations in the immune response also seem to contribute. Prior influenza A infection in mice primes for an exaggerated inflammatory response to subsequent pneumococcal infection (van der Sluijs *et al.*, 2004). Increased levels of IL-10 in this response likely contribute to increased susceptibility, as neutralization of this cytokine improves disease outcome (van der Sluijs *et al.*, 2004). *In vitro* exposure to both influenza A and the pneumococcus results in a synergistic inflammatory response from human middle-ear epithelial cells (Tong *et al.*, 2003). Microarray gene expression analysis of these cells following influenza A infection provides insight into the possible mechanisms behind this synergy (Tong *et al.*, 2004). For example, it is found that *tlr2* expression is upregulated by influenza A infection. This may make the cell more responsive to stimulation by pneumococcal peptidoglycan and LTA (Tong *et al.*, 2004).

It is therefore clear that the interaction of the pneumococcus with the innate immune system is greatly influenced by the presence of other organisms such as *H. influenzae* and influenza A.

Global analysis of host responses

The advent of microarray technology allows greater insight into the host-cell response to the pneumococcus. The response of the human monocytic cell line THP-1 has been assessed by microarray analysis following exposure to *S. pneumoniae* and an isogenic mutant lacking pneumolysin (Rogers *et al.*, 2003). After a three-hour exposure to the pneumococcus, expression differences were revealed in 182 host genes from the 4133 examined, illustrating the potential for large-scale expression changes induced by the pneumococcus (Rogers *et al.*, 2003). Of these 182 genes, 142 were responsive to pneumolysin, showing the dominant nature of this virulence factor in host responses. While this study will not be comprehensive in fully documenting the host response, it illustrates the complexity of the interaction between host cells and the pneumococcus. An important future challenge will be to understand the significance of these expression changes in the disease process.

Host gene-expression changes in an infection model have been investigated in a rat model of otitis media (Chen *et al.*, 2005). Twelve hours following pneumococcal challenge, 280 genes in the middle ear (effusion and mucosa) showed a greater than twofold change in expression compared with mock-infected controls. This represented approximately 24 % of the genes examined, again showing the ability of the pneumococcus to induce large-scale changes in host gene expression. Such data allow the pneumococcal response pathways to be mapped and the identification of previously unrecognized responses. For example, it has been found

that the transcription factor *fra-1*, implicated in bone proliferation, is upregulated during experimental otitis media. This provides a candidate mechanism to explain clinical features of otitis media involving the bone that are seen in both human patients and animals (Chen *et al.*, 2005). The global response to nasopharyngeal colonization has also been investigated in a mouse model (Nelson *et al.*, 2005). Upregulation of siderocalin, an iron-sequestering host-defence protein, has been noted in the nasal mucosa. How the pneumococcus causes this upregulation of siderocalin is unclear, with the effect still seen in mice deficient for either TLR2 or TLR4. Interestingly, this response could not be replicated *in vitro*, again showing the complexity of the immune response and the value of whole-animal systems (Nelson *et al.*, 2005). *S. pneumoniae* is resistant to siderocalin, and the upregulation of the latter may be advantageous to the pneumococcus by inhibiting potential competitors in the nasopharynx (Nelson *et al.*, 2005).

In addition to the host response, global analysis of bacterial gene expression *in vivo* has also been studied using models of meningitis and bacteraemia (Orihuela *et al.*, 2004). Together, these studies of host and bacterial response *in vivo* provide a platform to understand in molecular detail pneumococcal–host interactions from both perspectives.

Concluding remarks

Interactions between the pneumococcus and the innate immune system are multifaceted. Presented here are the recent advances in our understanding of these interactions highlighting the rapid progress in this area. Further understanding may eventually allow modulation of this interplay to aid therapy.

Acknowledgements

Work in the T. J. M. laboratory is supported by the Wellcome Trust, MRC, BBSRC, the Egyptian government and the European Union. Thanks to Dr C. E. Blue (University of Glasgow) for critical reading of the manuscript. Apologies to authors whose work could not be discussed or covered in full detail due to limitations on space.

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