How do microbes evade neutrophil killing?

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Summary

Many microbial pathogens evolved to circumvent the attack of neutrophils, which are essential effector cells of the innate immune system. Here we review six major strategies that pathogenic bacteria and fungi use to evade neutrophil defences: (i) turning on survival and stress responses, (ii) avoiding contact, (iii) preventing phagocytosis, (iv) surviving intracellularly, (v) inducing cell death and (vi) evading killing by neutrophil extracellular traps. For each category we give examples and further focus on one particular pathogenic microbe in more detail. Pathogens include Candida albicans, Cryptococcus neoformans, Yersinia ssp., Helicobacter pylori, Staphylococcus aureus, Streptococcus pyogenes and Streptococcus pneumoniae.

Neutrophils: how do they work?

The immune system protects the body from microbes that invade and harm the host. Neutrophils play a pivotal role in the resolution of microbial infections (Nathan, 2006). To fulfil this function neutrophils have a large antimicrobial arsenal at their disposal. The aim of this article is to review the strategies pathogenic bacteria and fungi have evolved to evade this arsenal.

In humans roughly 100 billion neutrophils enter and leave circulating blood every day (Gallin and Snyderman, 1999). Acquired or inherited neutropenia as well as neutrophil malfunction result in recurrent, life-threatening infections with bacteria and fungi (Kaufmann and Steward, 2005). This underscores the importance of neutrophils as a first line of defence in innate immunity. Paul Ehrlich first described neutrophils as polymorphonuclear leukocytes when new fixation techniques revealed lobulated nuclei and cytoplasmic granules containing host-defence molecules (Stengel et al., 1905). Elie Metchnikoff discovered the function of neutrophils and macrophages as migrating and phagocytosing cells (Metchnikoff, 1968). Neutrophils are the first immune cells recruited from the bloodstream to a site of inflammation. They are terminally differentiated cells synthesizing low amounts of RNA and protein and have a short life span of only a few hours. Neutrophils emerge from pluripotent haematopoietic stem cells in the bone marrow. Leaving the bone marrow, neutrophils are equipped with their antimicrobial arsenal to fight invading microbes. Upon contact, neutrophils engulf the microbes into a phagocytic vacuole, called a phagosome. Subsequently, intracellular granules fuse with the phagosome and discharge their contents to form a phagolysosome (Fig. 1A and B). In these phagolysosomes, microbes are killed by combination of non-oxidative and oxidative mechanisms (Nathan, 2006). The oxygen-independent effectors are stored in three different neutrophil granule subsets. The granules to be discharged first are the specific granules (also named secondary granules) and the gelatinase granules (also named tertiary granules). These granules contain an overlapping set of antimicrobial proteins, such as lactoferrin, lipocalin, lysozyme and gelatinase as well as metalloproteases important in tissue breakdown, which facilitates neutrophil migration and action (Fauschou and Borregaard, 2003). Next, the azurophilic granules (also named primary granules) are discharged. These peroxidase-positive granules contain small antimicrobial peptides, α-defensins, and seproci-
dins, antibiotic proteases, namely cathepsin G, proteinase 3 and neutrophil elastase. Azurophilic granules also harbour bacterial permeability increasing protein and myeloperoxidase (MPO) (Borregaard and Cowland, 1997).

The oxygen-dependent mechanism involves a non-mitochondrial generation of reactive oxygen species (ROS) and is known as the respiratory burst. A diverse set of ROS are formed during this process (Klebanoff, 2005). Upon activation of neutrophils the transmembrane and cytosolic subunits of the large NADPH-oxidase complex assemble at the phagosomal membrane and transfer electrons to molecular oxygen producing superoxide (O2·−). Superoxide spontaneously or through catalysis by superoxide dismutase (SOD) reacts to H2O2, which in
turn is the substrate of MPO to form hypochlorous acid (HOCl). MPO mainly produces hypochlorous acid in addition to hypobromous and hypoiodous acid. HOCl is the most bactericidal oxidant in neutrophils (Hampton et al., 1998). The chlorination of bacterial targets can inactivate, for example, iron-sulphur proteins, membrane proteins and the origin of replication site for DNA synthesis (Hampton et al., 1998). Furthermore, chloramines generated are also microbicidal.

Besides ROS reactive nitrogen species, mainly nitric oxide and peroxynitrite, are also potent antimicrobials. Mouse macrophages produce nitric oxide upon stimulation with cytokines. However, it is still unknown whether human neutrophils produce these substances and if so, what their contribution may be.

In addition, ROS have been implicated in the activation of granule proteases (Reeves et al., 2002; Ahluwalia et al., 2004). As the NADPH oxidase transfers electrons across the phagosomal membrane, the resulting charge depolarizes the membrane. Charge compensation is required to maintain function of the NADPH oxidase. It is controversial whether large conductance K$$^+$$-channels (BK channels) transport potassium ions into the phagosome to compensate, at least partially, for the charge differences (Reeves et al., 2002; Ahluwalia et al., 2004). Phagosomal ROS-production and K$$^+$$-influx may be crucial to solubilize the cationic granule proteases by releasing them from the anionic sulphated proteoglycan granule matrix. The solubilized proteases, in turn can become active. Blocking BK channels reduced killing of Staphylococcus aureus and Candida albicans, albeit functional phagocytosis and oxidase activity. This emphasizes an essential role of BK channels in the function of phagocytes. However, the existence of BK channels in neutrophils is in question and new results suggest that voltage-gated proton channels are crucial for charge compensation (Femling et al., 2006). Another study compared the superoxide-production, membrane potential and K$$^+$$-flux together with bacterial killing (Rada et al., 2004). At low levels of superoxide-production (at the onset of phagolysosomal killing) K$$^+$$-flux dominates and the phagosome is transiently alkalized. At later stages, protons take over, compensating pH and charge. Increasing superoxide-production to more than 20% of the maximum does not alter either the membrane potential or K$$^+$$-flux any further. The additional superoxide, however, dramatically increases the killing efficiency. Therefore, Rada et al. proposed that electrophysiological changes as well as ROS directly contribute to killing. Further research will determine the contribution of ion fluxes through different channels in neutrophil function.

In addition to the intracellular killing mechanisms described above activated neutrophils release granule proteins and chromatin. Together, these components form fibres called neutrophil extracellular traps (NETs). The presence of microbes is sufficient to induce the NET-release. NETs are able to bind and kill bacteria (Gram-positive and -negative) and a pathogenic yeast (Brinkmann et al., 2004; Urban et al., 2006). The major components of the NETs are DNA and associated histones (H1, H2A, H2B, H3 and H4). They contain smooth stretches of 15–17 nm in diameter, thought to consist of naked DNA and globular domains of around 25 nm where chromatin is present (Fig. 1C). The antimicrobial activity of histones is well established (Hirsch, 1958). Moreover, NETs contain granule proteins from azurophilic, specific and gelatinase granules. The NETs may possibly facilitate killing of microbes in two ways: (i) concentrate the antimicrobial arsenal to the site of infection and (ii) prevent the
spread of microbes from the initial site of infection. NETs are presumably formed after phagocytic killing mechanisms are exhausted because NET-formation is initiated after in vitro infection whereas neutrophils phagocytose microbes within the first minutes of contact (Urban et al., 2006).

A report investigating the transcriptional response of neutrophils to bacterial phagocytosis revealed that engulfment of pathogens results in neutrophil apoptosis (Kobayashi et al., 2003). The authors suggested that neutrophil apoptosis is crucial to resolve infections and that pathogen-induced premature apoptosis is an evasion mechanism for microbes. To distinguish between the contribution of neutrophil apoptosis and NETs in host defence, we first need to understand the mechanism of NET-formation in order to determine how the different fates of neutrophils contribute to their function.

**How do pathogens fight back?**

Pathogenic bacteria and fungi have evolved efficient strategies to outfox the weaponry of neutrophils. The main strategies can be divided into six categories: Launching a general survival response, avoiding contact (Fig. 2A), preventing phagocytosis (Fig. 2B), surviving inside the neutrophil (Fig. 2C), inducing cell death and (Fig. 2D), avoiding killing in NETs (Fig. 2E). In this review we will highlight one microbe out of each category, either a pathogenic bacterium or fungus, and will emphasize on recent findings.

### General responses by pathogens to neutrophils

Recent transcriptional analyses using whole genome microarrays of pathogens attacked by neutrophils give new insights into microbial responses (Rubin-Bejerano et al., 2003; Voyich et al., 2003; 2005; Fradin et al., 2005). A common feature of engulfed *C. albicans, S. aureus* and *S. pyogenes* is the upregulation of genes crucial for resistance to oxidative stress. Indeed, mutant strains of, for example, *C. albicans* (Marchenko et al., 2004) or *Pseudomonas aeruginosa* (Lau et al., 2005) lacking oxidative stress genes are less virulent. This highlights the importance of ROS in microbial killing and can be applied...
to both models discussed: (i) ROS kill microbes or (ii) ROS activate antimicrobial proteases.

*Candida albicans* is a commensal ascomycetous yeast that colonizes the skin and mucosae of healthy individuals. However, in immunocompromised patients *C. albicans* can cause severe life-threatening infections (Romani, 2004). One of its major virulence traits is the ability to reversibly switch morphology from singular budding cells to filamentous forms. *C. albicans* engulfed by macrophages can evade killing by inducing hyphal growth inside the phagosome (Mansour and Levitz, 2002) resulting in the destruction of the macrophage and growth inside the phagosome (Mansour and Levitz, 2002) resulting in the destruction of the macrophage and the escape of *C. albicans*. In contrast, *C. albicans* cannot form filaments in neutrophil phagosomes, and is therefore arrested in growth and eventually killed. However, *C. albicans* evolved a response that is triggered upon phagocytosis and renders the organism more resistant to neutrophil killing (Rubin-Bejerano *et al*., 2003). Upon contact with neutrophils, but not with other blood cells (Fradin *et al*., 2005), *C. albicans* increases expression of several oxidative stress genes, such as superoxide dismutases (SOD), catalases (CAT) and glutathione peroxidases (GPX) (Rubin-Bejerano *et al*., 2003) which neutralize the oxidative neutrophil arsenal. We may conclude from these studies that pathogens with a high oxidative stress tolerance are also more resistant to neutrophil killing. Therefore, scavenging ROS produced by the neutrophil NADPH oxidase is a virulence mechanism to evade host immune responses. This is consistent with the recent finding that the major pigment of *S. aureus*, a ROS-scavenger, increases survival in neutrophils and promotes virulence (Liu *et al*., 2005).

Transcriptional profiling of *C. albicans* also revealed that genes of the methionine and arginine biosynthetic pathway are induced upon phagocytosis by neutrophils (Rubin-Bejerano *et al*., 2003; Fradin *et al*., 2005). Growing *C. albicans* under amino acid deprivation mimicks this response, suggesting that neutrophil vacuoles are deficient in amino acids. Indeed, amino acid transporters in the phagosomal membrane, which is derived from the plasma membrane in neutrophils, might be (Rubin-Bejerano *et al*., 2003) oriented to pump amino acids out of the phagosome. Whether low concentrations of amino acids in the neutrophil phagosome is the trigger for the transcriptional activation of *C. albicans* remains to be determined.

Avoiding contact

Pathogens have two different strategies to avoid contact with neutrophils: First, they remain in regions inaccessible to phagocytes (Fig. 2A1). For example, *Listeria monocytogenes* induces its own uptake by epithelial cells (Pizarro-Cerda and Cossart, 2006). Second, many pathogenic bacteria and fungi are able to prevent recruitment of neutrophils to the site of infection (Fig. 2A2): For example, the fungus *Blastomyces dermatitis* avoids provoking an inflammatory response by reducing the production of tumour necrosis factor alpha (TNF-α), a proinflammatory cytokine, by host cells (Finkel-Jimenez *et al*., 2002). Thus, TNF-α-induced activation and recruitment of neutrophils and monocytes is inhibited. *S. aureus* secretes the chemotaxis inhibitory protein (CHIPS) that binds to the formyl peptide receptor and to the C5a receptor on neutrophils. This binding blocks signalling cascades which are activated in neutrophils upon contact with the bacteria and are crucial for neutrophil migration to the site of infection (de Haas *et al*., 2004).

*Cryptococcus neoformans*, a yeast-like, encapsulated fungus, also prevents neutrophil migration by secreting capsular components. *C. neoformans* can cause severe disseminated infections, such as cryptococcal meningitis, predominantly in immunocompromised individuals (Diamond and Erickson, 1982). Although sera of patients with disseminated cryptococcosis have high titres of *C. neoformans* products, the infiltration of leukocytes to the infection site is minimal. The secreted capsular component glucuronoxylomannan (GXM) reduces leukocyte chemotaxis in an infection model (Dong and Murphy, 1995). GXM activates microglia to produce interleukin-8, however, chemotaxis of neutrophils is still decreased (Lipovsky *et al*., 1998). This was explained by two findings: On the one hand, GXM reduces L-selectin (CD62L) on the neutrophil surface in humans with disseminated cryptococcosis (Dong and Murphy, 1996). As CD62L is essential for leukocyte extravasation from the bloodstream by mediating attachment to inflamed endothelial cells, neutrophil migration is impaired. On the other hand, GXM restrained neutrophil rolling on the endothelium (Ellerbroek *et al*., 2004). In addition, capsular components of *C. neoformans* can induce loss of tumour necrosis factor receptor (TNFR) on neutrophils. This loss of TNFR desensitizes neutrophils to TNF-α and is another strategy employed by the fungus to avoid contact with phagocytes (Dong and Murphy, 1996; Coen-jaerts *et al*., 2001). These findings demonstrate that while *C. neoformans* is susceptible to phagocytosis and killing of human neutrophils (Miller and Mitchell, 1991), it can evade neutrophils altogether by suppressing their migration.

Preventing phagocytosis

A very efficient strategy to overcome killing by neutrophils is to prevent engulfment (Fig. 2B). Pathogenic bacteria and fungi use three different approaches: they (i) use physical barriers, such as polysaccharide or poly-
 glutamate capsules, that prevent recognition by phagocytes, (ii) interfere with opsonization, for example, by precluding complement activation and (iii) inhibit the actin cytoskeleton required for engulfment.

Uropathogenic Escherichia coli e.g. employs the first approach. The capsular antigens O75 and K5 increase the resistance to phagocytosis (Burns and Hull, 1999). As an example of the second approach S. aureus secretes the complement inhibitor SCIN that decreases complement-mediated phagocytosis (Rooijakkers et al., 2005). The third approach is used by three pathogenic Gram-negative bacteria Yersinia pestis (bulbonic and pneumatic plaque), Yersinia pseudotuberculosis (gastroenteritis) and Yersinia enterocolitica (mesenteric adenitis). Upon contact of the bacteria with a host cell the bacterial type III secretion apparatus injects effector proteins into the cytoplasm of the host cell. In vivo, the main target cells for Yersinia effector proteins are dendritic cells, macrophages and neutrophils (Marketon et al., 2005). Four of those proteins YopE, YopH, YopT and YopO inhibit the actin cytoskeleton (Gruenheid and Finlay, 2003).

YopH and YopE both decrease phagocytosis by neutrophils (Grosdent et al., 2002) and increase virulence (Viboud and Bliska, 2005). YopH is a tyrosine phosphatase (Zhang et al., 1992). Tyrosine phosphorylation is involved in actin cytoskeletal signalling pathways. YopH-mediated dephosphorylation at the site of phagocytosis blocks a Ca$^{2+}$-influx which is crucial for actin rearrangements (Persson et al., 1999). YopE acts on Rho GTPases that are also involved in actin rearrangements (Black and Bliska, 2000; Von Pawel-Rammingen et al., 2000). In vivo, YopE predominantly targets the GTPase Rac-1 (Black and Bliska, 2000; Von Pawel-Rammingen et al., 2000; Aili et al., 2006) which is particularly implicated in the uptake of unopsonized bacteria (Gruenheid and Finlay, 2003). Notably, several strains carrying single amino acid substitutions in YopE are avirulent in mice. These mutants express and translocate higher amounts of YopE than parental strains suggesting that YopE may also regulate the level of effector translocation during infection (Aili et al., 2006).

The effectors YopT and YopO, which also target Rho GTPases, reduce neutrophil uptake (Grosdent et al., 2002) but are not crucial for virulence (Juris et al., 2002; Trulzsch et al., 2004). This might indicate that functionally redundant effectors exist. The regulation of phagocytosis by Yersinia effectors is likely to be even more complex. For example, a recent proteomic approach identified eight novel effector proteins, one of which, YspP, is a tyrosine phosphatase (Matsumoto and Young, 2006). Taken together, Yersinia evolved several effector proteins that inhibit phagocytosis and make it virulent.

Surviving inside the neutrophil

Many bacteria and fungi survive in neutrophils after phagocytosis (Table 1). Anaplasma phagocytophilum, like other Ehrlichia species use neutrophils as a host cell and evolved fascinating strategies to survive within this hostile environment. New findings about these Gram-negative bacteria causing tick borne fever-like illness are summarized in recent reviews (Carlyon and Fikrig, 2003; Dumler et al., 2005; Rikihisa, 2006).

Intracellular survival strategies can be categorized into (i) inhibition of phagosome lysosome fusion (Fig. 2C1), (ii) survival inside the phagolysosome (Fig. 2C2), and (iii) escape into the cytoplasm (Fig. 2C3).

Streptococcus pyogenes, a Gram-positive bacterium (Group A Streptococcus), is able to use each of these strategies. S. pyogenes is one of the most common human pathogens causing pharyngitis, impetigo, scarlet fever and also severe systemic disease (Cunningham, 2000). First, streptococcal M and M-like proteins are able to prevent degranulation as well as phagosomal fusion of azurophilic granules (Staali et al., 2006). Second, proteins H and M mediate survival of S. pyogenes inside the phagolysosome of neutrophils despite a strong oxidative burst following internalization (Staali et al., 2003). During infections S. pyogenes seem to use neutrophils to hide from immune responses. Neutrophils from infected murine spleens are sufficient to cause infections after inoculating naive mice (Medina et al., 2003). Third, the S. pyogenes capsule may enable the bacterium to escape the neutrophil phagosome into the cytoplasm as revealed by electron micrographs of infected neutrophils (Medina et al., 2003). The bacterium forms large capsules after incubation with host phagocytic cells resulting in increased virulence and resistance against phagocytic killing.

Another strategy that enables survival inside the phagolysosome is applied by Helicobacter pylori (Allen et al., 2005). The Gram-negative bacterium subverts the action of neutrophil NADPH oxidase. H. pylori colonizes the gastric mucosa of humans and causes gastritis, which in some cases can progress to gastric cancer. Although H. pylori induces a respiratory burst in neutrophils the bacterium can direct a large proportion of the ROS to the extracellular space (Allen et al., 2005). H. pylori is most likely able to redirect the assembly of the NADPH-oxidase complex to the plasma membrane and to exclude the phagosomal membranes that surround the bacterium. The underlying mechanism still needs to be elucidated.

Inducing cell death

Streptococcus pyogenes (Sierig et al., 2003; Miyoshi-Akiyama et al., 2005), Clostridium perfringens (Stevens
et al., 1987) and *S. aureus* secrete toxins which lyse neutrophils and other host cells. The presence of these toxins strongly correlates with the virulence of these strains (O’Reilly et al., 1986; Patel et al., 1987). *S. aureus* is a Gram-positive bacterium which is responsible for a wide variety of community- and hospital-acquired infections. This opportunistic pathogen has evolved several immune evasion mechanisms such as the secretion of pore-forming toxins (PFTs). *S. aureus* expresses two types of PFTs α-toxin, also known as α-haemolysin and bicomponent leukotoxins (Luk). Alpha-toxin forms homo-oligomers whereas bicomponent Luk have two distinct subunits that oligomerize into hexa- or heptameric forms (Foster, 2005). Four different types of bicomponent Luk exist in *S. aureus* the γ-toxins or γ-haemolysins, leukocidin E/D, leukocidin M/PV-like and Panton-Valentine leukocidin (PVL) (Menestrina et al., 2001). All *S. aureus* toxins apply a similar mode of action. The water soluble monomeric forms bind the plasma membrane of target cells, and oligomerize into a ring structure to form a transmembrane pore (Menestrina et al., 2001). The N-terminus of α-toxin is necessary to prevent the premature oligomerization of the complex in solution but is not required for oligomerization and pore-formation (Jayasinghe et al., 2006). Whereas α-toxin is present in most of the sequenced *S. aureus* strains only 1–2% express PVL (Peacock et al., 2002; Foster, 2005). However, PVL is found predominantly in strains causing severe skin infections and recurrent furunculosis (Prevost et al., 1995). Moreover PVL has been correlated to community acquired multiresistant strains causing necrotizing pneumonia (Gillett et al., 2002). PVL localizes to neutrophils and epithelial cells in the lungs of patients with necrotizing pneumonia (Genestier et al., 2005). In neutrophils the toxin induces either necrosis at high toxin concentration or apoptosis at low concentration (Genestier et al., 2005). The authors showed that PVL directly targets the mitochondria of neutrophils, activates Caspase-9 and hence causes apoptosis.

### Avoiding killing in NETs

In addition to phagocytosis and intracellular killing, neutrophils release NETs that capture and kill microbes in the extracellular space (Brinkmann et al., 2004; Urban et al., 2006). Nucleases can degrade NETs indicating that the chromatin functions as a scaffold and is a major component of the fibres. Interestingly, extracellular nucleases

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**Table 1.** Summary of known pathogenic bacteria and fungi that are able to resist neutrophil killing.

| Organism                                      | Evasion strategy | References                      |
|-----------------------------------------------|------------------|---------------------------------|
| **Gram-negative bacteria**                    |                  |                                 |
| Actinbacillus actinomycetemcomitans           | C, D             | Permpanich et al. (2006)        |
| Bordetella pertussis                          | C                | Steed et al. (1991)            |
| Brucella abortus                              | C                | Elzer et al. (1996)            |
| Burkholderia ssp. (B. pseudomallei, B. cenocepa) | A, C            | Bylund et al. (2006)          |
| Chlamydia ssp. (C. pneumoniae, C. trachomatis) | C                | Register et al. (1986)         |
| Ehrlichia ssp. (Anaplasma phagocytophilum)    | GR, C            | Rikihisa (2006)                |
| Escherichia coli                              | B                | Burns and Hull (1999)          |
| Francisella tularensis                        | C                | Lofgren et al. (1983)          |
| Haemophilus ssp. (H. somnus, H. influenzae)   | C                | Czuprynski and Hamilton (1985) |
| Klebsiella pneumoniae                         | B                | Domenico et al. (1994)         |
| Legionella pneumophila                         | C                | Horwitz and Silverstein (1981) |
| Neisseria gonorrhoeae                          | C                | Simons et al. (2005)           |
| Pseudomonas aeruginosa                        | GR, B, C         | Bayer et al. (1991)            |
| Rickettsia tsutsugamushi                       | C                | Rikihisa and Ito (1979)        |
| Salmonella ssp. (S. typhi, S. typhimurium)     | D                | Chiu and Ou (1999)             |
| Yersinia ssp. (Y. pestis, Y. enterolytica)     | B                | Viboud and Bliska (2005)       |
| **Mycobacteria**                              |                  |                                 |
| Mycobacterium ssp. (M. leprae, M. tuberculosis) | C                | Holzer et al. (1986)           |
| **Gram-positive bacteria**                    |                  |                                 |
| Bacillus anthracis                             | A                | During et al. (2005)           |
| Clostridium perfringens                        | A, D             | Stevens et al. (1987)          |
| Enterococcus (E. faecalis, E. faecium)         | C                | Rakita et al. (1999)           |
| Staphylococcus (S. aureus, S. epidermidis)    | GR, A, B, C, D   | Foster (2005)                  |
| Streptococcus (S. pyogenes, S. pneumoniae)    | GR, A, B, C, D, E| Voyich et al. (2004)           |
| **Fungi**                                     |                  |                                 |
| Blastomyces dermatitidis                       | A, C             | Schaffner et al. (1986)        |
| Cryptococcus neoformans                        | A, B             | Diamond and Erickson (1982)    |
| Histoplasma capsulatum                         | C                | Kurita et al. (1991)           |
| Paracoccidioides brasiliensis                  | C                | Schaffner et al. (1986)        |
| Sporothrix schenckii                           | C                | Schaffner et al. (1986)        |
| Trichosporon beigelii                          | B, C             | Lyman et al. (1994)            |

GR, general survival response; A–E, different categories of evasion strategies as displayed in Fig. 2.

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are found in several pathogenic bacteria including S. aureus (Cuatrecasas et al., 1967), Clostridium perfringens (Okumura et al., 2005) and S. pyogenes (Sumby et al., 2005). A streptococcal DNase has previously been implicated in disease progression (Sumby et al., 2005). This prompted two independent laboratories to investigate whether surface localized DNases of Streptococcus strains degrade NETs. S. pyogenes expresses the extracellular DNase Sda1 (Buchanan et al., 2006) and Streptococcus pneumoniae EnDA respectively (Beiter et al., 2006). Both groups showed that the streptococcal nucleases degrade NETs in vitro. In a skin infection model S. pyogenes wild-type strains are more virulent than sda1 knock out strains (Buchanan et al., 2006). Sda1 increases resistance against killing by human neutrophils and in whole mouse blood. Furthermore, sda1 mutants, not wild-type strains, are killed by human neutrophils when phagocytosis is blocked. Simultaneous administration of G-Actin, a specific inhibitor of DNases, prevents this effect (Buchanan et al., 2006).

Streptococcus pneumoniae is the leading cause of community-acquired pneumonia, with high morbidity and mortality. Human neutrophils do not kill S. pneumoniae although the NETs trap the organism. A mutant lacking endA infects the upper respiratory tract in mice but fails to disseminate into the lung and bloodstream (Beiter et al., 2006). Both reports establish streptococcal surface localized DNase as a bona fide virulence factor that degrades NETs. Extracellular nucleases from other bacteria, such as S. aureus or C. perfringens, are likely to degrade these fibres as well.

Concluding remarks

The interaction between neutrophils and pathogens remains a challenging subject. The host requires the action of neutrophils to fight invaders and the pathogens in turn must cope with neutrophil attacks in order to colonize the host. Microbial pathogens and hosts are in an arms race that mutually drives the evolution of the microbial evasion and survival mechanisms as well as that of the host immune system. We reviewed the evasion strategies applied by pathogenic bacteria and fungi showing that each of them promoted virulence of the respective organism. In particular, pathogens that have evolved several different modes to evade neutrophil killing, such as S. aureus and S. pyogenes, are wide spread and cause severe diseases, underscoring the essential role of neutrophils in innate immunity. By conceiving novel concepts of neutrophil evasion by microbial pathogens we are able to understand essential steps in innate immunity. This in turn has the potential to inspire new antimicrobial therapeutics as well as modulators of the inflammatory response.

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