OPINION

Can innate immunity be enhanced to treat microbial infections?

B. Brett Finlay and Robert E. W. Hancock

Innate immunity is a highly effective set of conserved mechanisms used by multicellular organisms to recognize and counter the constant threat of microbial infections. There is evidence to indicate that innate responses are key to controlling most infections, as well as contributing to inflammatory responses that are central components of disease. In addition to Toll-like-receptor-mediated effects, many other mechanisms are used to recognize and respond to microbial threats. Natural molecules such as CpG DNA and small cationic peptides trigger innate responses that help to control infection. This indicates there is potential to utilize such compounds to activate or enhance innate responses as antimicrobials. Harnessing this activity, without associated harmful inflammatory responses, is the main challenge.

The immune system is carefully crafted to provide protection from the potentially harmful effects of foreign agents, such as microorganisms, cells from other individuals and even our own damaged or transformed cells. Immunity and the field of immunology are often defined conceptually in the context of antigen-specific responses and include humoral and cellular adaptive immunity. However, another important area of immunity — innate immunity — is increasingly recognized as a central defence mechanism[1,2]. This is the main (and often only) form of immunity in less complex species, including insects and plants, which have no adaptive immune responses. Many organisms therefore function in the presence of potentially pathogenic microorganisms without possessing adaptive immunity. Innate immunity is clearly a crucial component of local immunity in more complex animals, including humans, whereas adaptive immunity represents an evolutionary addition to the overall immune strategy of higher organisms. Innate and adaptive immunity are interconnected in such animals and it is often hard to define the boundaries between the two, especially as they share many similar effector mechanisms. In the view of Janeway and Medzhitov[2], innate immunity involves events that occur almost immediately after exposure to a foreign microorganism, whereas a few hours after exposure, the animal host has shifted to early adaptive immunity. We would argue that innate immunity involves defence mechanisms that are not antigen specific and do not require rearrangement of certain receptor and immunoglobulin genes to generate the exquisite specificity that is associated with antigen recognition.

Indeed, with this broader definition, innate immunity is clearly related to the processes of acute and chronic inflammation as well as to such potentially harmful events as sepsis. There are several features of innate immunity that distinguish it from adaptive immunity. First, effector mechanisms, which function at a certain background level but are activated immediately after microbial exposure and can be further upregulated over a period of hours. Second, microbial recognition, which involves fixed receptors on the surface of certain cells and is followed by receptor-induced signalling but not receptor-gene rearrangement. Third, antigen-specific recognition is not involved, although effectors might be selective for particular types of foreign agents through recognition of molecular or structural motifs (sometimes called pathogen-associated molecular patterns). Fourth, a lack of self versus non-self discrimination that can lead to potential problems when the immune system is overstimulated, as host tissues can be damaged as part of the process. It is our thesis that some of these features, namely the rapid action, ability to be amplified and limited specificity of innate immunity, can be exploited for broader therapeutic benefit. Typical elements of innate immunity that have important roles in controlling infections are summarized in TABLE 1.

Many recent reviews have stressed the importance of a class of receptors known as Toll-like receptors (TLRs) in innate immunity. The original Toll receptor was found in *Drosophila* and is involved in the induction of a particular element of innate immunity, namely the antifungal peptide drosocin. It rapidly became apparent that elements of the *Drosophila* signalling pathway involving Toll receptors were related to mammalian pathways that converged on the nuclear translocation of a transcription factor called nuclear factor-κB (NFκB) (the *Drosophila* homologue is known as Rel) and the subsequent induction of the expression of many genes. Further work defined the mammalian TLR family as involving several receptors that recognize conserved molecules, many of which originate from infectious organisms. However, stimulation of TLRs is known to involve events that are both favourable, such as the induction of chemokines to attract phagocytes, and potentially detrimental, such as the induction of septic pro-inflammatory cytokines like tumour necrosis factor (TNF). Therefore, induction of the common pathway from Toll-like receptors to NFκB can be considered analogous to wielding a double-edged sword, one that can resolve infection but can also cause great harm.

Table 1 | **Typical elements of innate immunity involved in controlling infections**

General response	Effects
Pro-inflammatory response	NFκB mediated; activates many agents of inflammation; overstimulation can result in shock
Chemotaxis	Increased endothelial adhesion of phagocytic cells; cell migration to site of infection; diapedesis
Cationic host defence peptides	Increased production of cationic peptides stimulated by bacterial signalling molecules
Phagocytic cell activation	Increased intracellular killing in neutrophils and macrophages (both oxidative and non-oxidative mechanisms are enhanced); increased cytokine production
Extracellular killing mechanisms	Complement activation; antimicrobial peptide secretion; enhanced iron chelation; production of degradative enzymes
Infection containment	Clot formation via fibrinogen activation
Wound repair	Fibroblast growth and adherence; angiogenesis
Activate adaptive immune responses	B- and T-cell activation, often via dendritic cells

Innate immunity is complex

There has been a marked increase in our knowledge of how TLRs recognize microbial antigens and signal a variety of mechanisms that are central to the control of infection. However, it is also becoming apparent that there are other receptors and pathways that have important roles in the innate response, which is sometimes overshadowed by the intense interest in TLR signalling. In addition to TLRs, there is a recently discovered class of cytoplasmic molecules, known as nucleotide-oligomerization domain (NOD) proteins (which include NOD1 and NOD2). These proteins recognize microbial motifs (portions of peptidoglycan) generated by intracellular organisms[3]. Like TLRs, NODs activate NFκB pathways and generate inflammatory responses, thereby providing an innate mechanism with which to respond to the presence of intracellular pathogens. Mutations in *NOD2* are correlated with increased chronic intestinal inflammation and Crohn's disease, indicating that this molecule might be involved in the control of intestinal microorganisms and inflammation. A general signalling pathway downstream of the TLRs has been defined (FIG. 1) and involves, amongst others, the important adaptor protein myeloid differentiation protein 88 (MyD88). However, as is often the case, there is increasing evidence that there are alternate downstream pathways. Recently, LPS2, a key transducer of a MyD88-independent signalling pathway downstream of TLR3 and TLR4, was identified[4]. Similarly, in a *Listeria monocytogenes* murine infection model, induction of macrophage chemotactic protein-1 (MCP-1), a key chemokine required for monocyte recruitment, is MyD88 independent and MyD88 deficiency does not impair monocyte recruitment, although it can impair monocyte activation[5]. In addition, MyD88 is not needed to control mycobacterial infections in mice[6] and murine macrophage activation by *Mycobacterium tuberculosis* does not require MyD88 (REF. 7). In *Drosophila*, where Toll was first recognized, the MyD88 equivalent (known as Pelle) is needed to control fungal and Gram-positive bacterial infections, but not Gram-negative bacterial infections[8]. Wells *et al.*[9] used array analysis of murine macrophage responses in several genetic backgrounds to show that many loci other than TLR4 control responses to lipopolysaccharide (LPS). In another recent study[10], it was shown that mindin, an extracellular matrix protein, mediates a strong innate (inflammatory) response to several bacterial surface ligands that is independent of TLRs. Collectively, these studies indicate that there are other pathways and mechanisms used by the innate system to detect and respond to microbial threats.

Perhaps the strongest evidence for the existence of non-TLR-mediated innate responses is provided by a recent study of three children lacking interleukin-1 (IL-1) receptor associated kinase 4 (IRAK4) — a molecule that is central to TLR-mediated signalling[11] (FIG. 1). As expected, these patients do not activate NFκB or mitogen-activated protein kinases (MAPKs) and do not produce inflammatory cytokines such as IL-1 in response to known TLR agonists. However, although in their first 4–5 years of life these patients suffered from recurrent infections, these infections were caused by relatively few types of microorganisms — mainly by extracellular pyogenic bacteria such as *Streptococcus pneumoniae* and *Staphylococcus aureus*). Furthermore, these infections became less frequent with age and by 6–11 years of age the children were generally healthy, even in the absence of treatments. Significantly, this indicates that innate responses other than TLR-mediated pro-inflammatory cytokines can control infectious encounters. Similarly, another patient with mutations in IRAK4 failed to mount a significant inflammatory response in an aseptic inflammation model[12], which indicates that the TLR pathway is needed for normal inflammatory responses, so humans must have alternate mechanisms to enable such patients to survive. The existence of such patients indicates that innate immunity involves elements that are independent of TLR signalling, and also that classical inflammatory responses, such as production of NFκB and IL-1, are not essential to controlling infections — an unexpected, but important conclusion.

It therefore becomes apparent that there must be mechanisms for limiting the duration of an innate immune response, especially the acute inflammatory response. Each day we are exposed to hundreds of thousands of microorganisms, including potential pathogens, so we clearly have the ability to prevent such microorganisms from progressing to infections. Yet, at the same time we remain tolerant to the large number of microorganisms that represent our normal flora and coexist with our own cells without inducing extreme inflammation[13]. TLRs, NODs, and other receptors are not designed to be 'pathogen specific', but rather to detect common microbial signatures or families of structurally similar molecules. Therefore, innate immunity must include elements that can provide protection while suppressing (or not inducing) harmful effects, and these mechanisms must be designed to restore the system to a baseline level, representing a system of homeostasis in which the normal flora are not immunostimulatory. As discussed above, it is now evident that inflammatory events are not indispensable for the control of infections. This raises the possibility that, if the appropriate innate responses can be activated, it might be possible to activate protection against pathogenic organisms without hyperstimulation of potentially harmful inflammatory responses.

Beneficial innate immune stimulation

There are several indications that innate immunity and the resulting inflammation can be stimulated, leading to altered outcomes of infectious diseases. For example, interferon-γ (IFN-γ) has been used to treat refractory mycobacterial infections[14], and IFN-α, used with the antiviral drug ribivirin, is a standard treatment for hepatitis C infections. The TLR7

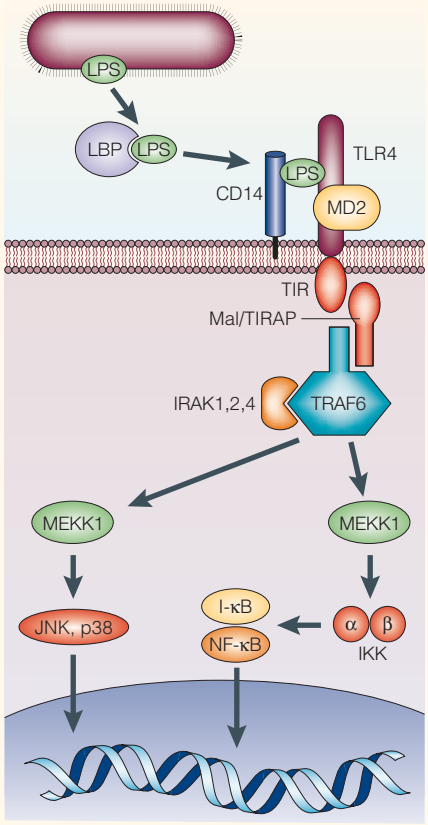

Figure 1 | **Generalized major signal transduction pathway of TLR4.** Lipopolysaccharide (LPS) forms a complex with lipopolysaccharide-binding protein (LBP), which binds to cell surface CD14. This then forms a complex with Toll-like receptor 4 (TLR4) and MD2. On the cytoplasmic side of the membrane, TLR4 interacts with MyD88 (myeloid differentiation protein 88) and Mal/Tirap (myeloid differentiation protein 88 adaptor-like/Toll-IL-1-receptor-domain-containing adaptor protein), which then activates a cascade involving interleukin-1 receptor associated kinase (IRAK), tumour-necrosis-factor-receptor-associated factor 6 (TRAF6), and eventually nuclear factor-κB (NFκB), which results in transcriptional activation of inflammatory genes. IKK, I-κB kinase; JNK, Jun N-terminal kinase; MEKK, mitogen-activated protein kinase/ERK kinase.

agonist imiquimod has been approved for use in several viral infections, such as human papillomavirus (which causes condyloma and common warts) and molluscum contagiosum[15]. Monophosphoryl lipid A (MLA) is a lipid A analogue that stimulates TLR4. This compound has proven to be an effective adjuvant for use in vaccines, but is also under development independently as an immunomodulatory agent[16]. The above examples indicate that, in certain cases, enhancing the cytokine and inflammatory responses downstream of TLR-mediated signalling can be used to treat infections. However, given the detrimental effects that are often associated with inflammation, the question remains whether it is possible to uncouple unwanted inflammation from beneficial antimicrobial effects. Although this concept is counter to most that deal with inflammation, two natural classes of molecules, cationic peptides and CpG oligonucleotides, provide indications that such an approach could be used to counter microbial infections.

Small cationic peptides as modulators of the innate immune response. Small cationic peptides[17–19] are abundant in nature and have been described as 'nature's antibiotics' or 'cationic antimicrobial peptides'. Closer examination of the properties of such molecules has indicated that a more appropriate term is 'cationic host defence peptides' because they modulate the innate immune response and not all have significant antimicrobial activities under conditions that reflect their *in vivo* locations[20]. These cationic peptides are 12–50 amino acids long with a net positive charge of +2 to +9, which is due to an excess of basic arginine and lysine residues, and approximately 50% hydrophobic amino acids[18]. They fold into amphiphilic structures with hydrophobic and hydrophilic (charged) faces. However, as well as having a wide variety of sequences within a given structural group, the secondary structures of these peptides vary considerably, and include small β-sheets stabilized by disulphide bridges, amphipathic α-helices and, less commonly, extended and loop structures. The relatively rapid sequence divergence of these peptides has been assumed to be driven by adaptive evolution to counter microbial adaptation and diversity[21,22]. This diversity is thought to provide increased variability to respond to rapidly evolving microorganisms. However many non-antibiotic host proteins, such as those that are involved in reproduction, immunity and host defences[23], have diverged rapidly, so it is by no means certain that antimicrobial activity drove this evolution. It is also possible that the more conserved regions are used for moderating innate defences (see below), whereas the diversity provides altered mechanisms for direct antimicrobial activity. To understand the evolutionary changes in these molecules more needs to be known about the molecular mechanisms that underlie peptide actions.

There is much evidence that peptides are important in protection against microorganisms (TABLE 2). For example, peptides such as human LL-37 and its mouse homologue CRAMP have been shown to be critical in clearing infections such as necrotic skin infections caused by group A streptococcus[24]. Expression of human defensin-5 (HD-5) in Paneth cells in transgenic mice protects against salmonellosis[25], whereas mice lacking β-defensin-1 demonstrate delayed clearing of *Haemophilus influenzae* from the lung[26]. Numerous animal model studies have indicated that both natural and synthetic cationic peptides are able to protect against infections in animal models, despite the fact that their protease-sensitive nature dictates that they would be quite labile *in vivo*, with half-lives that could be measured in minutes.

In the appropriate body setting, certain peptides have meaningful antibiotic activity[17,20]. For example α-defensins are present in the azurophilic granules (lysosomes) of neutrophils. Following phagocytosis of bacteria by neutrophils and subsequent fusion of the resulting phagosomes with these lysosomes, bacteria are exposed to α-defensins at milligram per millilitre concentrations, which is sufficient to mediate non-oxidative killing and/or hypersensitization of bacteria to lysosomal enzymes. Similarly, CRAMP, the murine homologue of cathelicidin LL-37, mediates intracellular killing of *Salmonella* species within macrophages[27]. In addition, β-defensins that are found in intestinal crypts at estimated concentrations of 25 mg ml^{-1} are able to directly kill microorganisms. Certain other natural peptides, such as horseshoe crab polyphemusin and pig protegrin, are very active as antimicrobials and even at low concentrations can be active *in vivo*. Significantly, cationic peptide variants have been developed clinically as topical antibiotics with proven efficacy[28].

However, this is not the case for all natural host defence peptides as the antimicrobial activity of these peptides is often extrapolated from *in vitro* measurements at high concentrations and under non-physiological, low-ionic-strength conditions. By contrast, divalent cations (such as Mg^{2+} or Ca^{2+}), which are at concentrations of 1–2 mM and present in almost every body fluid, will reduce or completely eliminate the antibiotic activity of many of the natural peptides. Moreover, peptides such as LL-37 and β-defensins are found at mucosal sites in the body at concentrations of less than 2 μg ml^{-1}. When this is compared with the minimal inhibitory concentration (MIC) of LL-37 for *Escherichia coli* in nutrient medium, which is more than 32 μg ml^{-1}, it suggests that other mechanisms of host defence that do not involve direct killing are likely to have a role at these sites. It is worth noting that these host defence mechanisms (discussed below) can retain activity in whole blood containing 3 mM divalent cations[29].

Table 2 | **Known effects of human cationic peptides**

Effect	Peptide	Possible Role in Immunity
Induction during inflammation or infection, and at wound sites and blisters	LL-37; selected α- and β-defensins	Synthesis triggered by situations that might involve infection; possibly relates to anti-inflammatory or infection-suppressing activities
Selectively suppresses expression of LPS-induced genes in a macrophage cell line	LL-37	Antisepsis/anti-inflammatory; reduces cytokine expression; might function as a feedback inhibitor of inflammation
Anti-endotoxin activity	LL-37; BPI, CAP18, CAP- and CAP57-derived peptides	Protection against overstimulation of pro-inflammatory cytokines
Causes signalling of host cells of innate immunity through MAP kinase pathways	LL-37, β-defensin 3	Modulation of the activity and/or gene expression of these host cells
Act directly as chemoattractants for neutrophils, monocytes, mast cells and T helper cells	LL-37; selected α- and β-defensins	Recruitment of cells of innate immunity to the infection site
Stimulation and release of neutrophil and monocyte chemokines from host cells	LL-37; selected α- and β-defensins	Recruitment of cells of innate immunity to the infection site
Directly stimulates transcription of multiple genes in cells of innate immunity	LL-37	Modulation of the activity and/or differentiation state of phagocytic and epithelial cells
Mast cell degranulation leading to histamine release	β-defensin-2 (not -1), α-defensins, histatins, LL-37	Increase in blood vessel permeability (vasodilation)
Increased production of integrins involved in chemotactic responses	LL-37, α-defensins	Promotion of non-opsonic phagocytosis
Promotion of fibroblast chemotaxis and growth, re-epithelialization of wounds	LL-37, α-defensins	Involvement in tissue and wound repair
Activation of endothelial cells to promote angiogenesis	LL-37	Production of new blood vessels promotes wound healing
Activation of murine mast cell killing of group A streptococcus	LL-37	Promotes bacterial clearance
Promotion of adherence of some bacteria to epithelial cells	Defensins	Localizes bacteria
Influence on development of adaptive immunity	α- and β-Defensins and LL-37	Promotion of systemic immune defences if innate immunity fails

LPS, lipopolysaccharide; MAP, mitogen-activated protein.

Collectively, these data indicate that such peptides have a critical role in innate defences. What is exciting are indications that, in addition to their potential antimicrobial activity, these peptides have activities that favour resolution of infection, while actually reversing potentially harmful inflammation.

Anti-endotoxic activity of cationic host defence peptides. It has been known for some time that polymyxin B can bind to endotoxin and has anti-endotoxin activity, as evaluated by the ability to suppress the production of pro-inflammatory cytokines (including TNF-α, IL-1 and IL-6) and protect against endotoxin-mediated lethality in animal models. Small cationic peptides derived from polymyxin show similar anti-endotoxic activity[30], even in the absence of antimicrobial activity. It was subsequently shown that short (10- or 11-mer) cationic peptides derived from a human neutrophil granule protein known as bactericidal permeability increasing factor (BPI or CAP57) were able to neutralize the ability of *E. coli* 055:B5 endotoxin to cause gelation of a *Limulus* amoebocyte lysate[31]. Again, the abilities of these molecules to kill bacteria and neutralize endotoxin were clearly distinct. Cationic peptides from the neutrophil protein CAP18 (especially the amino-terminal 37 amino acids that subsequently became known as LL-37) had anti-endotoxic activity, including an ability to protect mice against LPS lethality[29,32], as did cationic peptides from *Limulus* anti-lipopolysaccharide factor and lipopolysaccharide-binding protein[33]. Many other studies demonstrating anti-endotoxin activities of cationic peptides have since been published.

Gough *et al.*[34] demonstrated similar activities in the synthetic cationic antimicrobial peptides CEMA and CEME (also called MBI-28 and MBI-27, respectively). The *in vivo* protection provided by these peptides correlated with an ability to neutralize the LPS-induced hyperexpression of TNF-α in murine macrophages. However, although these peptides bound potently to LPS, it was shown that CEMA and polymyxin B could neutralize TNF-α production, even when added to the murine macrophages 60 minutes after LPS. These data indicated for the first time that neutralization of endotoxin responses by cationic peptides might be more complicated than merely reflecting LPS binding or neutralization. This is perhaps not surprising as other peptides are not polycationic and do not seem to bind well to LPS, including anti-inflammins[35] and certain neuroactive peptides, are able to protect against endotoxin in animal models.

A second line of evidence that argues against a mechanism of suppression of endotoxaemia that is based solely on binding and neutralization of LPS by the cationic host defence peptides was provided by microarray experiments[29,36]. Interaction of the synthetic insect-derived peptide CEMA with LPS led to selective modulation of gene responses induced by LPS in the mouse macrophage RAW264.7 cell line. More than 40 genes that were upregulated by LPS, including pro-inflammatory cytokines like IL-1β and IL-15, were suppressed to varying extents by CEMA, whereas another 16 LPS-upregulated genes were completely unaffected. Although there are several possible explanations for this selective effect, these data do not favour the explanation of binding and neutralization of LPS by this peptide. As an alternative explanation we proposed that cationic peptides such as CEMA act directly on macrophages to regulate signalling pathways, and that this differentially affects the ability of LPS to upregulate the

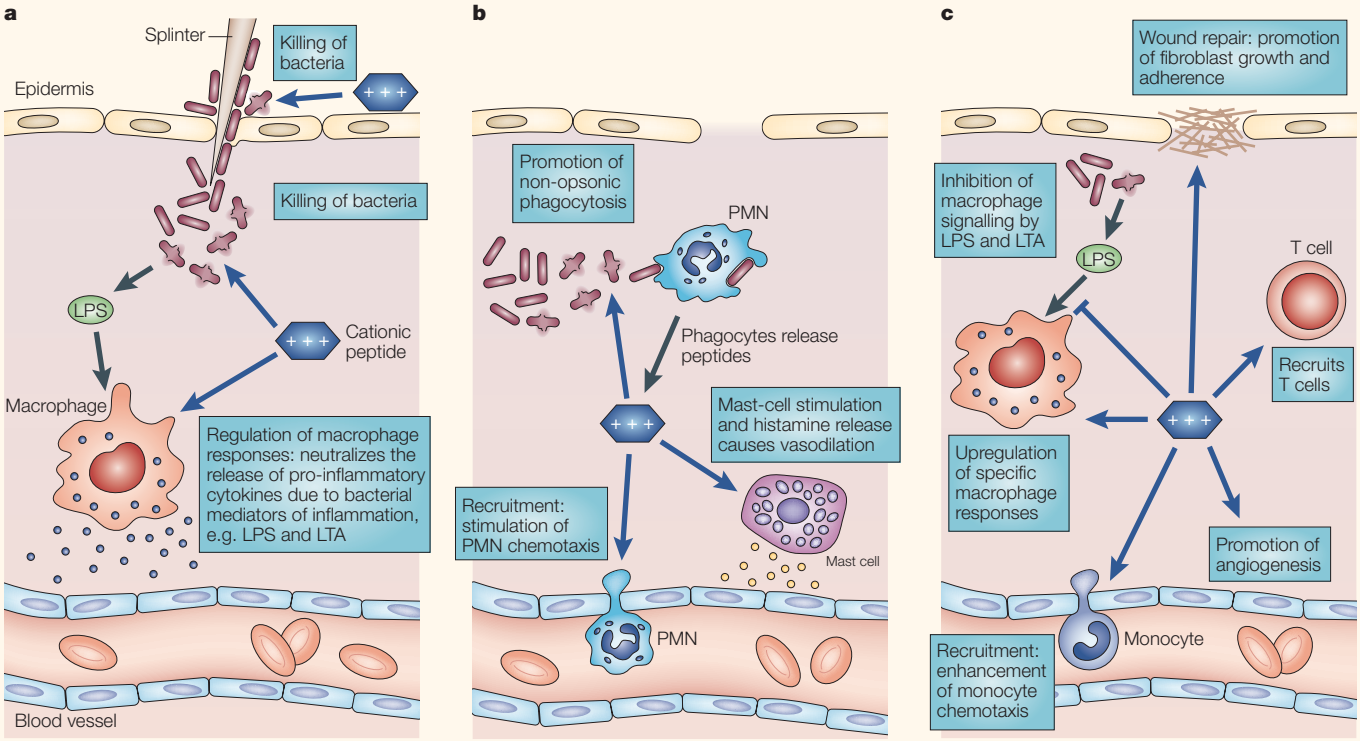

Figure 2 | **Effects of cationic peptides on innate responses.** Cationic peptides have been reported to be involved in many aspects of innate host defences associated with acute inflammation (**a**, **b**). If acute inflammatory responses are insufficient to result in bacterial clearance, then chronic inflammation and adaptive immune responses are initiated (**c**). Although individual peptides have been shown to have the specific functions shown in the figure, no single peptide has been shown to carry out all functions. Therefore, the overall scheme presented is a composite of these separate effects. LPS, lipopolysaccharide; LTA, lipoteichoic acid; PMN, polymorphonuclear leukocytes. Modified with permission from REF. 38 © (2000) Elsevier.

expression of selected genes; consistent with this was the demonstration that CEMA could stimulate the expression of a large and distinct set of genes in macrophages[37]. It was subsequently shown that the human host defence peptide LL-37 had a similar ability to upregulate some genes and downregulate others[29]. A working hypothesis is that the products of a subset of these peptide-upregulated genes are able to suppress endotoxic responses that lead to production of pro-inflammatory cytokines (which, as mentioned above, are known to be activated by LPS and suppressed by these peptides). Suppression might involve a direct impact on signal transduction along the TLR to NFκB pathway or a direct anti-inflammatory mechanism. Although there is no published proof in support of this hypothesis, other data that we have obtained using more comprehensive arrays are consistent with this explanation.

Modulation of protective innate immunity by peptides. There is excellent evidence that host defence peptides have activities that correspond to many aspects of innate immunity usually associated with acute inflammation and host defence[38–41] (FIG. 2). For example, host defence peptides have the ability to act directly as chemoattractants for neutrophils, monocytes, mast cells and T-helper (T_H) cells, and/or to stimulate the release of neutrophil and monocyte chemokines from host cells, which result in recruitment of cells of the innate immune system to the infection site. They can also stimulate mast-cell degranulation, which leads to histamine release and a consequent increase in blood-vessel permeability (vasodilation). Third, they can increase production of integrins that are involved in chemotactic responses and promote non-opsonic phagocytosis. Fourth, they inhibit the lysis of fibrin clots by tissue plasminogen activator, thereby reducing the spread of bacteria. Fifth, they can induce the release of inducible nitrous oxide synthetase to increase the rates of bacterial killing. Sixth, they can stimulate tissue and wound repair through promotion of fibroblast chemotaxis and growth. Seventh, by inhibiting certain proteases such as furin and cathepsin they can inhibit tissue injury. Finally, they can promote angiogenesis in endothelial cells and wound healing. Although these responses would be considered pro-inflammatory, with certain exceptions they are quite distinct in representing only a modest subset of typical pro-inflammatory responses to infectious agents and their signalling molecules like LPS and lipoteichoic acid (LTA), and cationic host defence peptides actually suppress the stimulated production of pro-inflammatory cytokines like TNF-α and IL-6.

The mechanisms by which these multiple events are mediated by cationic host defence peptides are becoming clearer. As mentioned above, peptides like CEMA and LL-37 stimulate the expression of many genes in macrophages, including some innate-response genes[29,37]. Consistent with these gene-array experiments, LL-37 directly upregulates certain chemokines, including MCP-1 and IL-8, and chemokine receptors, such as CXCR4, CCR2 and IL-8 receptor-B, in macrophages, the mouse lung, human A549 epithelial cells and whole human blood without stimulating the pro-inflammatory cytokine TNF-α[34]. Other upregulated genes include certain growth factors and growth-factor receptors, as well as oncogenes. One possibility is that LL-37 causes differentiation of cells of innate immunity to allow them to more effectively perform anti-infective functions in innate immunity. In this regard, LL-37 is a potent modulator of dendritic-cell differentiation[42,43].

Also consistent with this view, it was recently shown that LL-37 induces the activation of the MAPKs, extracellular signal-regulated kinase (ERK)1/2 and p38 in human peripheral blood-derived monocytes and human airway epithelial cell lines[36,44]. Activation of these kinases has multiple impacts on the effector cells of the immune response, including chemokine production and cellular activation, proliferation and differentiation[45]. Furthermore, signalling was synergistically promoted by the growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF)[44]. Bowdish *et al.*[44] confirmed that exposure to LL-37 led to activation of the transcription factor Elk-1 — which is downstream of, and activated by, phosphorylated ERK1/2 — which in turn led to the upregulation of various Elk-1-controlled genes and the transcription and secretion of IL-8. Furthermore, inhibition of either the p38 or ERK1/2 kinases led to a reduction in LL-37-induced IL-8 secretion and the inhibition of transcription of various chemokine genes (such as IL-8, MCP-1 and MCP-3). The receptor upstream of the MAPK activation event (which is proposed to be the epidermal growth-factor receptor[36]) was found to be different from the receptor that has been proposed for the direct chemokine activity of LL-37 — formyl peptide receptor-like-1 (REF. 46). Other studies using mast cells[47] are consistent with there being a diversity of LL-37 receptors on different cell types, apparently with different downstream consequences.

As discussed above, the peptide LL-37 is chemotactic and directly stimulates the production of certain chemokines and chemokine receptors on host epithelial cells and monocytes. Defensins also use multiple cellular receptors, which allows them to act directly as chemokines[48] as well as to stimulate the production of chemotactic cytokines, including IL-8, epithelial neutrophil-activating peptide (ENA)-78, MCP-1 and GM-CSF in bronchial epithelial cells[49]. This might reflect an inter-relationship between the functions of the classical chemokines and the cationic host defence peptides because 17 of 30 chemokines studied so far have *in vitro* antimicrobial activity[50] that may or may not be important *in vivo* (owing to the relatively high chemokine concentrations and low salt concentrations that were used in this study). The authors found no obvious sequence relationship between these two groups of protein, but proposed that these functional similarities reflect the topological formation of a large, positively charged patch. As observed with LL-37, murine β-defensin-2 has been reported to influence differentiation of dendritic cells[42].

Although it is difficult to determine the exact contribution of each of the many activities of cationic host defence peptides, the emerging picture is that these compounds generally enhance host defences at the site of infection, while dampening harmful inflammatory effects mediated by TNF-α and related TLR-induced cytokines.

CpG oligonucleotides. Immunostimulatory oligodeoxyribonucleotides (ODNs) that contain the nucleotide pair CpG (called CpG-ODNs) are strong immunomodulators that have shown promise in animal models for treating infections[51]. Unlike eukaryotic DNA, bacterial CpG-ODN motifs contain unmethylated cytosine, which provides a bacterial signal that the host can use to activate innate immunity. Unmethylated CpG-ODN sequences are recognized by TLR9 (REF. 52) and trigger a variety of innate responses. These include the production of pro-inflammatory cytokines, such as IL-6, and the chemokine IFN-γ-inducible protein 10 (IP-10) (REF. 53), which are responses that are normally associated with TLR activation. Also, dendritic, phagocytic and natural killer (NK) cells can become activated in response to CpG[54]. However, different CpG-ODNs induce a range of inflammatory responses, which indicates that such sequences might be specific to human responses to individual pathogens (for example, compared with responses in mice)[55]. In addition to effects on innate immunity, CpG can impact on adaptive immunity, acting as adjuvants that induce strong T_H1-type responses that are usually associated with cellular immunity (FIG. 3).

So far, almost all of the studies on CpG efficacy in animal model infections have focused on intracellular infections[51]. For this reason, it has been suggested that a crucial mediator is IFN-γ, which is possibly generated through the induction of a T_H1 response, although it is not clear whether an antigen-specific response is required for protection, as treatment prior to challenge with the infectious organism tends to lead to protection. The first use of CpG-ODNs in mouse model infections was for the treatment of *Leishmania major* infections[56]. Studies showed that the CpG-ODN could be administered before, during, or 20 days after a lethal infection and still protect the mice. Other studies have shown that mice can also be protected against the malarial parasite, *Plasmodium yoelii*. However, the results with a variety of viral infections are somewhat more equivocal with responses that ranged from protection through attenuation and even acceleration of disease[51,57].

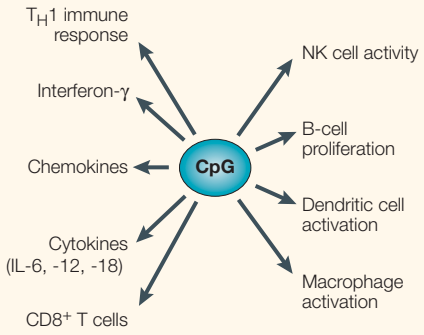

Figure 3 | **Effects mediated by CpG oligonucleotides.** Many effects have been reported for CpG oligonucleotides, with most of these being associated with enhancing an adaptive immunity T-helper 1 (T_H1) response, including interferon-γ production. It has not been clearly established how these effects are linked through Toll-like receptor 9 (TLR9) recognition of CpG DNA. IL, interleukin; NK, natural killer.

CpG oligonucleotides have also been used prophylactically to prevent *Francisella tularensis* and *L. monocytogenes* murine infections via a mechanism that was found to be nonspecific but dependant on lymphocytes[58]. Similarly, CpG-ODN was able to protect against *Edwardsiella tarda* infections of olive flounder, a result that correlated with priming of the oxidative burst in phagocytes[59].

Recently, it has been shown that CpG oligonucleotides can decrease the bacterial load of *Helicobacter pylori* in the stomachs of mice[54]. *H. pylori* is an extracellular pathogen that adheres to the gastric mucosal surface leading to where it causes inflammation, leading to gastric ulcers and gastric carcinomas in humans. Intragastric administration of CpG-ODN caused an increase in the concentration of chemokines, such as macrophage inflammatory protein 1α (MIP-1α), MIP-1β, RANTES and IP-10. Similarly, CpG-ODNs have been used to protect chickens against extracellular *E. coli* infections[60]. CpG-ODNs were equally effective whether delivered locally or systemically, indicating that these molecules function through circulating factors and cells, rather than by localized actions. Collectively, this work indicates that CpG oligonucleotides may impact on aspects of innate immunity other than the T_H1/IFN-γ pathways that are associated with intracellular pathogens.

Harnessing the innate response

The ability to enhance or augment the innate immune response clearly represents a potentially powerful way to prevent or treat infections. As the innate response is not pathogen-specific, such stimulatory agents could have a broad spectrum of activity, and

biotechnology companies have begun to look at using cationic peptides and CpG oligonucleotides as potential enhancers of the innate response to treat infectious diseases. However, an important concern regarding this strategy for boosting innate responses is that mimicking the mechanisms by which natural pathogens stimulate innate immunity would lead to harmful inflammation and consequent host damage. Sepsis, which is a classic example of 'overactive' innate responses to pathogens and/or pathogen molecules, already afflicts more than 600,000 individuals and causes 120,000 deaths in the United States annually.

Central to the design of therapeutic agents that modulate innate responses will be greater knowledge of the various innate responses to microorganisms, and the classification of these responses as beneficial or harmful. The application of genomics and array studies to the interaction of host cells with microorganisms are allowing scientists to better define the pathways that are involved in innate responses. This knowledge should enable the discovery of agents with activities that selectively promote protection while not inducing, or even blocking, sepsis. Nature has provided significant clues as to the existence of agents that boost innate immunity, as discussed above for cationic peptides and CpG-ODN. A significant concern for treating bacterial diseases with agents like CpG-ODN, is that if this agent works through the TLR-signalling pathway, the stimulation of which can induce the overproduction of pro-inflammatory cytokines[53], such agents could in fact reinforce sepsis; however, this effect might be limited in cases where the cellular receptor is not broadly distributed throughout the body, as is probably true for TLR9, the CpG-ODN receptor. The fact that cationic host defence peptides tend to be anti-inflammatory (see above) provides hope that selective upregulation of innate immunity is feasible.

An important concern regarding the continuing effectiveness of conventional antibiotics is the development of resistance. It is worth considering whether compounds that boost innate immunity would also induce resistance. This could be problematic as it might select for microorganisms that have enhanced abilities to overcome normal defences. Successful pathogens have already evolved methods that allow them to overcome or bypass normal host innate defences, providing they are present in sufficient numbers[61,62]; indeed, these mechanisms presumably make them successful pathogens. However, pathogens often require multiple mechanisms to bypass the assortment of defences that constitute the immune system. By contrast, most opportunistic pathogens do not possess such sophisticated methods of overcoming host defences, but instead infect immunocompromised hosts.

Unlike antibiotics, drugs that boost innate immunity would not put a direct selective pressure on microorganisms. However, there is the potential that they might be able to counteract one or more innate mechanisms. It could be argued that complete resistance is unlikely as most pathogens have some susceptibility to the collective mechanisms of innate immunity (that is, at low numbers they do not infect animals or humans). Further lessons can be learned from vaccine resistance, as the concepts are somewhat analogous, involving 'enhanced' adaptive immunity rather than innate immunity. Although microorganisms can become resistant to vaccines (by eliminating, varying or coating surface antigens), vaccine resistance levels in bacteria are generally lower than antibiotic resistance levels. It could be argued that viral resistance has a greater potential to become a significant issue, as many viruses (such as RNA viruses) mutate their genomes rapidly, and viruses have an absolute requirement for host cells for replication. However, there are few, if any, viruses that are resistant to strong innate modulators such as IFN-γ.

In addition to the use of antimicrobial agents for preventing or treating infections, drugs that boost the beneficial aspects of innate immunity are prime candidates for potential broad-spectrum antimicrobial agents that could be deployed in a bioterror scenario[63]. These drugs could provide protection from multiple pathogens, which would potentially include antiviral, antibacterial, antifungal and antiparasitic activities. An additional benefit would be that the biological agent would not need to be identified in advance of treatment or prophylaxis. However, until such drugs are developed and tested, the ability to overcome highly infective pathogens (which presumably have many mechanisms to overcome innate defences) remains untested.

In conclusion, studying innate immunity will allow us to understand how the body responds to microorganisms and controls infections. Innate responses are processes that are controlled by natural compounds that have evolved an effective way to allow complex organisms to live in a world in which microorganisms are abundant. By understanding these processes, there is significant potential for exploiting these mechanisms to provide new ways of controlling infectious diseases.

B. Brett Finlay and Robert E. W. Hancock are at the University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.
Correspondence to B.B.F.
e-mail: bfinlay@interchange.ubc.ca

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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SCIENCE AND SOCIETY

Salmonella, stress responses and food safety

Tom Humphrey

The ability of *Salmonella* to survive in the food chain is due, in part, to its ability to respond effectively to environmental changes. It is unlikely that *Salmonella* will ever be eradicated from the food chain — the results from laboratory research, such as investigations of the response of *Salmonella* to different stresses, must therefore be translated into improved intervention strategies for food producers and consumers.

Salmonella is an important food-borne pathogen throughout the world. It has been reported that, worldwide, there are more than 1.3 billion cases of human salmonellosis annually, with three million deaths[1]. These estimates are from 1995 and present a global picture. More recent data estimate that there are 1.3 million cases of salmonellosis in the United States each year, with 600 deaths (see Salmonellosis disease information in the online links box). Other recent work indicates that the mortality rates of individuals who have been infected with *Salmonella* are three times those of non-infected individuals in the year after infection[2]. There are also substantial financial costs associated with *Salmonella* infection. There are therefore clear public-health benefits and economic gains to be made by being able to control the spread of this pathogen in the food chain.

The scientific community is on the threshold of knowing more about *Salmonella* than ever before. The complete genome sequence of *Salmonella enterica* serovar Typhi (*S.* Typhi)