

## REVIEW

## Mechanisms of bacterial pathogenicity

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Pathogenic bacteria utilise a number of mechanisms to cause disease in human hosts. Bacterial pathogens express a wide range of molecules that bind host cell targets to facilitate a variety of different host responses. The molecular strategies used by bacteria to interact with the host can be unique to specific pathogens or conserved across several different species. A key to fighting bacterial disease is the identification and characterisation of all these different strategies. The availability of complete genome sequences for several bacterial pathogens coupled with bioinformatics will lead to significant advances toward this goal.

Infectious diseases are the leading cause of death worldwide. Not only are new infectious diseases emerging, but the re-emergence of deadly infectious diseases, and the increasing prevalence of antimicrobial resistant strains, present a formidable threat to public health and welfare. Recently, significant evidence has emerged which indicates that markedly different microbial pathogens use common strategies to cause infection and disease. For example, many diverse bacterial pathogens share common mechanisms in terms of their abilities to adhere, invade, and cause damage to host cells and tissues, as well as to survive host defences and establish infection. A diagrammatic overview of some of these commonalities of infection appear to be related to the acquisition of large blocks of virulence genes from a common microbial ancestor, which can be disseminated to other bacteria via horizontal transfer. The horizontal transmission of large blocks of virulence determinants is also directly attributable to the constant emergence of new strains of bacterial pathogens, many of which are resistant to multiple antibiotics. Indeed, antibiotic resistance of bacterial pathogens has emerged as one of the most important issues facing critical care practitioners. A more thorough comprehension of the common themes in microbial pathogenicity is essential to understanding the molecular mechanisms of microbial virulence and to the development of novel vaccines and other therapeutic agents for the treatment and prevention of infectious diseases. While it is beyond the scope of this review to discuss in-depth details of the molecular mechanisms of bacterial pathogenesis (the reader interested in such detail is referred to a recent review by Finlay and Falkow<sup>1</sup>), this review focuses on a number of common mechanisms used by bacterial pathogens to cause infectious disease.

## CAPSULE

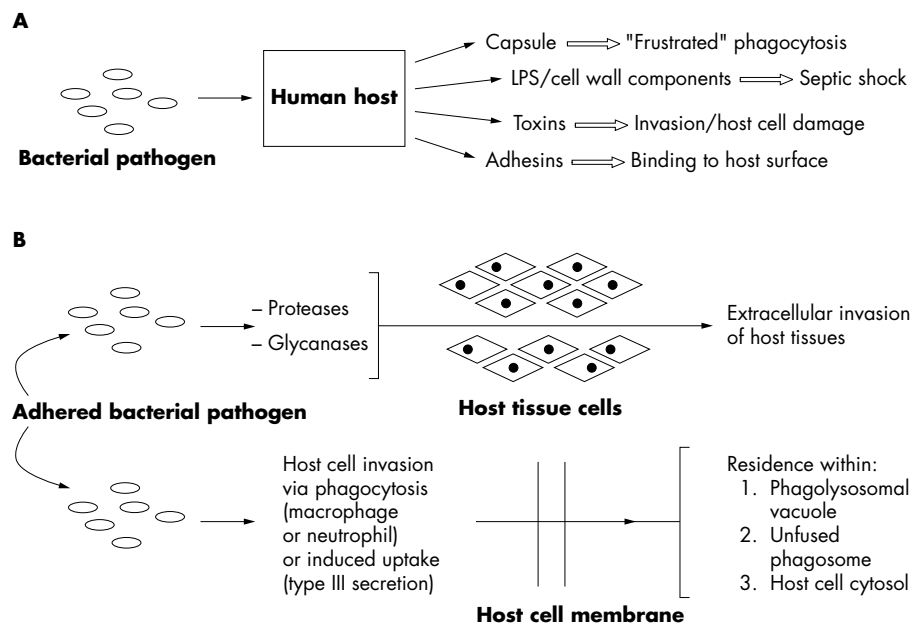
Some bacteria overproduce and excrete copious amounts of high molecular weight polysaccharides, also called exopolysaccharides, when isolated from clinical samples. This extracellular sugar coating is termed capsule. Different species of bacteria utilise diverse sugars to produce the capsule. Capsule production is one of the major virulence factors utilised by bacteria to evade clearance from an infectious site. Specifically, the capsule provides bacteria with protection from the host immune response as well as antibiotics. Some capsules have also been shown to have immunomodulatory effects. The capsule protects bacteria from phagocytosis by not allowing opsonising antibodies to be recognised by phagocytic host defence cells (for example, macrophages and neutrophils). This “frustrated phagocytosis” leads to enhanced inflammatory response as the macrophages and neutrophils produce more inflammatory cytokines in an attempt to clear the bacteria. The enhanced inflammatory response leads to increased tissue damage as more neutrophils and macrophages are recruited to the site of infection.

The most notorious species of bacteria that produce capsules are *Streptococcus pneumoniae* (pneumococcus), *Neisseria meningitidis* (meningococcus), and *Pseudomonas aeruginosa*. The pneumococcus capsular polysaccharide has been identified as one of its primary virulence factors. The pneumococci utilise 24 biosynthetic genes to produce their capsule.<sup>2</sup> There are at least 90 different capsular types, although only a subset of 23 types causes more than 90% of invasive disease worldwide.<sup>3</sup> Differences in capsular polysaccharide chemical structure determine the meningococcal serogroups.<sup>4</sup> Meningococci of serogroups B, C, Y, and W-135 express capsules composed entirely of polysialic acid or sialic acid linked to glucose or galactose,<sup>4,5</sup> while the capsule of serogroup A is composed of *N*-acetyl mannosamine-1-phosphate.<sup>4</sup> The *P aeruginosa* capsule is composed of alginate (acetylated mannuronic and guluronic acid). The biosynthetic enzymes for alginate have been elucidated and much is known about the genetic regulation for the overproduction of alginate. The unique characteristic of the *P aeruginosa* capsule is that all *P aeruginosa* strains have the genetic capability to produce alginate but it is most frequently found in cystic fibrosis isolates.<sup>6</sup> Though these capsules have different chemical composition and immunomodulatory effects, they serve to protect the

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**Figure 1** An overview of bacterial mechanisms for pathogenicity. (A) Upon encountering a human host, a bacterial pathogen may illicit several host responses and use a variety of mechanisms to evade the host defences. The bacterial components that interact with the host include: (1) capsules that act to "frustrate" phagocytosis and protect the pathogen from macrophage and neutrophil engulfment, (2) lipopolysaccharide (LPS) and cell wall components which can cause septic shock, (3) toxins that can serve to damage host cells and aid invasion, and (4) adhesins which facilitate binding of the pathogen to host surfaces. The degree to which these various mechanisms play a part in the pathogenesis of an infection depends on the bacterial species or strain, the site of pathogen entry, the immune status of the host and other similar factors. (B) Once adhered to a host surface, a bacterial pathogen may further invade host tissues. Pathogens may "burrow" further into a tissue by expressing and secreting proteases and glycanases that digest host extracellular matrix proteins and polysaccharides. In addition, a pathogen may also invade the host tissue cells and gain access to the intracellular environment. This can be facilitated by the natural phagocytosis mechanisms of macrophages and neutrophils or by induced uptake where the pathogen signals the host cell to engulf adhered bacteria. A common strategy for pathogens to induce uptake is the use of a type III secretion system which injects bacterial signalling proteins into the host cell. Within the host cell, the pathogen may reside within a phagolysosome (a phagosome which has fused with a lysosome), a phagosome which has not fused with a lysosome, or within the host cell cytosol.

bacteria from the host inflammatory response (that is, complement activation and phagocyte mediated killing).

### CELL WALL

Bacteria can be divided into two major groups based on differences in cell wall structure: Gram positive and Gram negative bacteria. The cell wall of both Gram positive and Gram negative bacteria contain toxic components that are potent virulence factors and have central roles in the pathogenesis of bacterial septic shock, a frequently lethal condition that involves collapse of the circulatory system and may result in multiple organ system failure. Unlike the conventional toxins described below, which are proteins produced by the bacteria and are usually secreted into the surrounding medium (exotoxins), the toxic components of the prokaryotic cell wall are distinct structural components that are not released appreciably into the extracellular medium until cell death and lysis of the bacteria. Ironically, antibiotics used in the treatment of microbial sepsis may release increased amounts of toxic bacterial cell wall components, and thus exacerbate treatment and negatively impact the eventual outcome for the host. It is believed that Gram negative and Gram positive bacteria may activate a common pathway of events that lead to septic shock.<sup>7-9</sup>

Septic shock is the result of the combined action of cytokines, complement components, and coagulation cascade components. Bacterial cell wall derived constituents can induce the host to produce or activate these mediators. Indeed, the proximate triggering event of septic shock is the release of lipopolysaccharide (LPS) or other toxic bacterial cell wall components into the circulation. Bacterial LPS (also known as endotoxin) is a large amphophilic molecule embedded in the outer membrane of Gram negative bacteria and is usually

considered to be the principal component responsible for the induction of septic shock that often accompanies severe infections with these microbes. The primary receptor for LPS is CD14, a cell surface marker of macrophages.<sup>10</sup> Lipid A, the toxic portion of the LPS molecule, causes release of numerous host proinflammatory cytokines and activates the complement cascade and the coagulation cascade. Recent studies suggest that toll-like receptors, inflammatory cytokines, eicosanoids, free radicals, macrophage migration inhibitory factor, signal protein kinases, and transcription factors, all play an important part in the pathobiology of Gram negative mediated septic shock (reviewed in Das<sup>11</sup> and Guha and Mackman<sup>12</sup>); also see "Interaction of pathogens with the innate immune system", below).

While endotoxin mediated events are clearly important in the host reaction to Gram negative infections, Gram positive bacteria can also induce septic shock and are increasingly recognised as major contributors to nosocomial sepsis. Gram positive bacteria do not have endotoxin, but the presence of these bacteria in tissues provokes an inflammatory response that is similar to that triggered by Gram negative LPS. Also, Gram positive bacteria in the bloodstream can cause the same type of septic shock symptoms as Gram negative bacteria. The same cytokines elicited by LPS are released and the same types of physiological effects are seen. This is, in part, because the peptidoglycan fragments and teichoic acids found in the Gram positive cell wall elicit many of the same physiological responses as LPS in the infected host. In Gram positive bacteria, peptidoglycan and teichoic acids (polymers of sugar alcohol phosphate) are the main potentiators of septic shock. The toxic cell wall components of both Gram positive and Gram negative bacteria act largely via the initiation of an inflammatory response through the stimulation of monocytes and macrophages and the subsequent release of proinflammatory

cytokines, especially tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 (reviewed in Verhoef and Mattson<sup>9</sup>). In addition, both endotoxin and peptidoglycan can activate the complement cascade, which induces the release of TNF- $\alpha$  from monocytes and induces aggregation of polymorphonuclear neutrophils and pulmonary vasoconstriction. Thus, regardless of whether a bloodstream infection is caused by Gram positive or Gram negative bacteria, the signs and symptoms of infection are similar.

Bacteria frequently implicated in septic shock include Gram negative microbes such as *Escherichia coli*, *P. aeruginosa*, and meningococci, and Gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and streptococci.<sup>13</sup> Interestingly, the aforementioned group of Gram positive bacteria have emerged as the most prevalent cause of hospital acquired infections, and as such, play a significant part in nosocomial sepsis (reviewed in Verhoef and Mattson<sup>9</sup>).

Toxic components of the cell wall of Gram negative and Gram positive bacteria are central mediators in the development of septic shock, an often fatal condition which is the result of the combined action of cytokines, complement cascade, and coagulation cascade components.

## TOXINS

Toxins are analogous to biological weapons in that these are proteinaceous or non-proteinaceous molecules produced by bacteria to destroy or damage the host cell. Examples of non-proteinaceous toxins are LPS (endotoxin) for Gram negative organisms and teichoic acid for Gram positive organisms (see "Cell wall" section above). Proteinaceous toxins (exotoxins) are generally enzymes which are delivered to eukaryotic cells by two different methods: (1) secretion into the surrounding milieu or (2) direct injection into the host cell cytoplasm via type III secretion systems or other mechanisms (see "Invasion" section below). Bacterial exotoxins can be roughly categorised into four major types based upon their amino acid composition and function: (1) A-B toxins, (2) proteolytic toxins, (3) pore forming toxins, and (4) other toxins.<sup>1</sup>

Several different species of bacteria contain A-B toxins including *P. aeruginosa*,<sup>14</sup> *E. coli*,<sup>15</sup> *Vibrio cholerae*,<sup>16</sup> *Corynebacterium diphtheriae*,<sup>17</sup> and *Bordetella pertussis*.<sup>18</sup> A-B toxins have two components: the A subunit which possesses the enzymatic activity; and the B subunit which is responsible for binding and delivery of the toxin into the host cell. The enzymatic activity of the A portion of A-B toxins ranges from proteolytic activity (for example, tetanus and botulinum) to ADP-ribosylating activity (for example, cholera, pertussis, diphtheria and *P. aeruginosa* exotoxin A).<sup>19</sup> Despite the range of enzymatic activity of the A portions of the numerous A-B toxins, there is a conserved nicotinamide adenine dinucleotide binding portion in A subunits suggesting that there may be a common evolutionary ancestor.<sup>20</sup>

Proteolytic toxins break down specific host proteins leading to some of the characteristic clinical manifestations of the disease. Examples of proteolytic toxins include: botulinum from *Clostridium botulinum*, tetanus from *Clostridium tetani*,<sup>21</sup> elastase,<sup>22</sup> and protease IV<sup>23</sup> from *P. aeruginosa*. The targets for the botulinum and tetanus toxins are synaptobrevins which prevent release of neurotransmitters resulting in different types of paralysis. These two toxins also differ in their sites of infection, botulinum is ingested and causes flaccid paralysis in peripheral nerves, whereas tetanus is found in deep wounds and results in spastic paralysis through the central nervous system. Elastase and protease IV of *P. aeruginosa* break down cellular matrix proteins allowing spread of the infection. Elastase is an important virulence factor for *P. aeruginosa*

pneumonia whereas protease IV is important in pseudomonal corneal infections.<sup>22, 23</sup> It is becoming apparent that the mode and location of toxin delivery affects the clinical symptoms manifested by the infected patient.

Membrane-disrupting toxins are found in a number of bacterial species and form a pore in the host cell membrane, which ultimately leads to cell lysis. There are a growing number of pore forming toxins included in the RTX family (named for a repeat arginine (R) threonine (T) X motif within each toxin) found in many Gram negative pathogens. Although the general mechanism of pore formation and sequences are conserved in the RTX family, the target cell specificities vary. The RTX family of toxins additionally share a common method of delivery (type I secretion).<sup>24</sup> Many Gram positive bacteria contain a sulfhydryl activated cytolysin. The best characterised among these is the listeriolysin O that is necessary for the escape of *Listeria monocytogenes* from the phagosome.<sup>25</sup>

In addition to the A-B toxins, others include: immunoglobulin A (IgA) protease-type proteins,<sup>26</sup> heat stable toxins that activate guanylate cyclase,<sup>27</sup> and toxins that modify the host cell cytoskeleton.<sup>28-31</sup> The common theme emerging from the study of these bacterial toxins is that bacteria are able to utilise many different methods to disrupt host cell signalling pathways and structural integrity to establish and maintain infection. We are currently beginning to acquire understanding of the molecular mechanisms involved in the action of these toxins. The good news is that there are a limited number of major toxin families that display common structural and biochemical motifs which may be exploited for future therapeutic development and these may be effective against multiple organisms.

## ADHESINS

A key step in the host-pathogen interaction is adherence of the pathogen to host surfaces. These surfaces include skin, mucous membranes (oral cavity, nasopharynx, urogenital tract), and deeper tissues (lymphoid tissue, gastric and intestinal epithelia, alveolar lining, endothelial tissue). Numerous mechanical forces produced by the host act to wash microbes from these surfaces: saliva secretion, coughing, sneezing, mucous flow, peristalsis, and blood flow. A common trait of microbial pathogens is the expression of factors that bind to molecules on various host tissue cells and render the microbe resistant to these mechanical washing forces. Once bound or "adhered" to a specific host cell surface, the pathogen is then able to initiate its specific biochemical processes that will result in disease including proliferation, toxin secretion, host cell invasion, and activation of host cell signalling cascades.

Microbial adherence factors are called adhesins and can be made from polypeptides (proteins) or polysaccharides (carbohydrates or sugars). Protein adhesins are separated into two groups: fimbrial and afimbrial. Fimbriae (also known as pili) are appendages that protrude as hair-like structures from the bacterial surface and are composed of proteins that are tightly packed into an array shaped like a helical cylinder. A single protein usually serves as the major fimbrial subunit, however other protein subunits also play structural roles at the tip and the base. Frequently, the fimbrial tip serves to bind the host receptor. Gram negative bacterial pathogens in particular rely on fimbriae for adherence. Examples include *E. coli* (for both urinary tract infections and gastroenteritis), *V. cholerae*, *P. aeruginosa*, and *Neisseria* species.<sup>32-34</sup> Afimbrial adhesins refer to proteins that serve as adherence factors, but do not form the long, polymeric fimbrial structure. The afimbrial adhesins generally mediate more intimate contact with the host cell that occurs over a shorter range than with fimbriae. Gram negative (*Yersinia pseudotuberculosis*, enteropathogenic *E. coli*, *Neisseria* spp), Gram positive (*Staphylococcus* spp, *Streptococcus* spp) and mycobacterial pathogens express afimbrial



adhesins.<sup>32 33 35–38</sup> Polysaccharide adhesins are usually components of the bacterial cell membrane, cell wall, or capsule. The teichoic acids found in the cell envelopes of Gram positive bacteria (refer to the “Cell wall” section of this article) serve as adhesins for *Staphylococcus* spp and *Streptococcus* spp.<sup>13</sup> The polysaccharides found in the capsule of *Mycobacteria* spp (glucan and mannan) are also recognised by host cell receptors (complement receptor 3 and mannose receptor) to promote adherence.<sup>39</sup>

Though the receptor-ligand interactions that occur to promote adherence can be divided into two general groups, protein-protein and protein-carbohydrate interactions, it is important to be aware of the variety of targets that microbes use for host receptors. The molecules that serve as host receptors for microbes include membrane-spanning proteins, surface immunoglobulin, glycolipids, glycoproteins, and extracellular matrix proteins (such as fibronectin and collagen).<sup>1 34 36 40</sup> In at least one case (enteropathogenic *E coli*), the pathogen injects its own protein receptor into the host cell.<sup>41</sup> Once in the host cell membrane, the receptor then binds to an afimbrial adhesin on the pathogen cell surface for adherence. It is also important to note that it is common for a single pathogen to express (and utilise) more than one adhesin. This strategy occurs in all types of bacterial species (Gram negative, Gram positive, and mycobacterial).<sup>1 13</sup> A current focus of research in antimicrobial therapy is the development of vaccines or drugs to block the adherence step in the infection cycle.<sup>42 43</sup>

## INVASION

Once adhered to a host surface, some pathogens gain deeper access into the host to perpetuate the infection cycle. This pathogenic principle, termed invasion, can be divided into two types: extracellular and intracellular. Extracellular invasion occurs when a microbe breaks down the barriers of a tissue to disseminate in the host while remaining outside of host cells. This is a strategy used by group A  $\beta$ -haemolytic streptococcus and *S aureus*.<sup>13</sup> These species secrete several enzymes that degrade host cell molecules: hyaluronidase (cleaves proteoglycans in connective tissue), streptokinase and staphylokinase (breaks down fibrin clots), lipase (degrades accumulated host oils), and nuclease (digests released RNA and DNA). The haemolysins (which punch holes in host cells) expressed by these species lyse not only erythrocytes but other cell types as well and may also contribute to their spread in host tissues. *Pseudomonas aeruginosa* secretes an enzyme, elastase, which degrades extracellular molecules and aids tissue invasion associated with keratitis, burn tissue necrosis, and cystic fibrosis.<sup>13</sup> Extracellular invasion allows these pathogens access to niches in tissues where they are able to proliferate, disseminate to other sites in the body, express toxins, and initiate inflammatory responses. There is a growing body of evidence that suggests that extracellular invading pathogens may also enter host cells and use both the extracellular and intracellular pathways during infection.<sup>37 44 45</sup>

Intracellular invasion occurs when a microbe actually penetrates the cells of a host tissue and survives within this environment. A number of Gram negative, Gram positive, and mycobacterial pathogens have been shown to have the ability to enter host cells,<sup>1 37 38 46</sup> and both phagocytic and non-phagocytic cell types can serve as targets for invasion. Some pathogens have an obligate intracellular lifecycle which absolutely requires a mammalian cell for growth. These include *Chlamydia* spp, *Rickettsia* spp, and *Mycobacterium leprae*.<sup>13</sup> Other pathogens are facultatively intracellular, using their ability to enter and survive within host cells as a means of proliferation or spreading to other tissues. A major advance in bacterial pathogenesis in recent years has been the identification of genes that allow pathogens to invade host non-phagocytic

cells. Remarkably, these invasion genes, present in several different pathogens, were found to encode an evolutionarily related type III protein secretion pathway that serves to inject signalling proteins from the microbe into the host cell. The injected proteins then activate host cell signalling pathways that cause the host cell to internalise the microbe. These entry mechanisms are well characterised in *Salmonella* spp and *Shigella* spp.<sup>32 47 48</sup> A common outcome of type III secretion signalling is the rearrangement of host cell actin such that the cytoskeleton is recruited to engulf the invading microbe. Both *Salmonella* and *Shigella* engage actin regulatory proteins, called Rho GTPases, to “switch on” the actin rearrangement pathway to form nodes of actin underneath the invading pathogen.<sup>32 48</sup> This type of interaction highlights the phenomenon of biochemical crosstalk between host and pathogen that is essential for penetration of host cells.

## INTRACELLULAR LIFESTYLES

Several bacterial pathogens have evolved to survive and replicate within host cells after invasion. The range of host cell types in which pathogens can survive include non-phagocytic cells (such as epithelial and endothelial) and professional phagocytes (such as macrophages and neutrophils). The ability to survive and replicate inside of phagocytic cells is particularly remarkable since these cells possess mechanisms to destroy ingested bacteria. These killing mechanisms include the production of reactive oxidative intermediates, the lowering of pH of bacteria-containing vacuoles, and the activation of degradative proteases. The strategies that bacteria use to avoid killing via these mechanisms are becoming increasingly well-characterised.<sup>1 49 50</sup>

There are three general intracellular niches in which pathogens reside: within an acidic, hydrolytically competent phagolysosomal vacuole; inside a vacuole which has not fused with a lysosome; and in the host cell cytosol. *Coxiella burnetii* is an example of a pathogen which is able to reside in the toxic environment of a phagolysosomal vacuole, and it has been shown that low pH is required to initiate its intracellular replication.<sup>1</sup> *Mycobacterium* spp, *Salmonella* spp, *Legionella pneumophila*, and *Chlamydia trachomatis* are included in the group which reside in non-lysosomal vacuoles. The vacuoles which are occupied by these pathogens are referred to as “specialised” or “remodelled” as they are usually morphologically different than other cellular vacuoles and contain a characteristic combination of surface markers.<sup>1 50</sup> *Shigella flexneri*, *L monocytogenes*, and *Rickettsia rickettsii* are pathogens which reside in the host cell cytosol.<sup>1 50</sup> These bacteria share a common strategy of enzymatically degrading the surrounding vacuole and spreading intracellularly via use of the host cell cytoskeleton.

Bacteria which survive intracellularly may replicate and spread to cells in the local area of infection or migrate to other areas of the body. *Chlamydia* and *Rickettsia* lyse the host cell membrane, releasing infectious bacteria which attach to and invade adjacent cells.<sup>13</sup> In addition to host cell lysis, *Shigella* and *Listeria* utilise a pathway of cell-to-cell spread which involves extension of the infected cell into an adjacent cell.<sup>13</sup> Invagination occurs where the infected cell has protruded into the adjacent cell, followed by membrane fusion and formation of a bacteria-containing vacuole in the adjacent cell. Bacteria residing in macrophages and neutrophils may use these cells as vehicles to spread systemically via the blood or lymphatic circulatory systems. *Salmonella typhi*, *Yersinia* spp, and *Brucella* spp are thought to move between tissues in this manner.<sup>13</sup>

Intracellular bacteria are particularly problematic in certain diseases. Certain intracellular infections can persist for years and require extensive antibiotic therapy, with *Mycobacterium tuberculosis* infection being a classic example. A major focus of current research is the identification and characterisation of

the molecular virulence factors that intracellular bacteria use to occupy this niche.<sup>51, 52</sup>

## REGULATION OF VIRULENCE FACTORS

The success of a microbe during pathogenesis relies on its ability to sense and respond to a myriad of environments during infection of the host. This requires the use of a repertoire of genetic functions on the part of the microbe which are independently regulated in response to environmental signals encountered inside the infected host. The regulation and timing of expression of virulence factors is very important for most pathogenic bacteria, as they encounter different micro-environments during the natural course of infection, each of which requires rapid adaptation to the new environment to allow the pathogen to colonise, survive, and grow within the host. Bacteria accomplish the intricate regulation of virulence factors by the use of a number of common motifs. This review will focus on two of the major regulatory control mechanisms used by pathogenic bacteria to control the expression of virulence genes: alternative sigma factors and the coordinate regulation of two component signal transduction systems.

### Sigma factors

Sigma factors are protein subunits of bacterial RNA polymerases (the enzymes that synthesise RNA from a DNA template), and control the initiation of transcription at the promoter sequence (unique sequences that define the start of a gene). Accordingly, sigma factors are a major regulator of prokaryotic gene expression. It is well known that bacteria use different sigma factors to control the initiation specificity at different promoters, including those promoters whose genes encode virulence factors. In particular, the alternative sigma factors RpoS ( $\sigma^{38}$ ) has been shown to regulate the expression of genes in response to stationary phase, nutrient deprivation, and oxidative and osmotic stress.<sup>53</sup> These are environments which are physiologically relevant to those encountered by many microbial pathogens during the natural course of infection. The RpoS sigma factor has been shown to be important for virulence in a number of bacterial pathogens, including *Salmonella typhimurium*,<sup>54</sup> *E coli*,<sup>55</sup> *P aeruginosa*,<sup>56</sup> and *L pneumophila*.<sup>57</sup>

Other alternative sigma factors involved in prokaryotic virulence gene regulation include RpoE ( $\sigma^{24}$ ), a sigma factor which responds to periplasmic stress and has been shown to be important for the virulence of the enteric pathogen *S typhimurium*<sup>58</sup>; RpoN ( $\sigma^{54}$ ) and AlgU which regulate the mucoid phenotype in *P aeruginosa*<sup>59</sup>; RpoH ( $\sigma^{32}$ ), a heat shock sigma factor which is important in the regulation of virulence in *Vibrio cholerae*<sup>60</sup>; and sigma F, which affects flagellar expression in the respiratory pathogen *Bordetella bronchiseptica*.<sup>61</sup>

### Two component systems

Two component regulatory systems consist of two proteins involved in the expression of virulence determinants. Typically, these systems are composed of: (1) a sensor protein that is embedded in the bacterial membrane which "senses" different physiological conditions of the bacterial cell and (2) a response regulator which usually binds to the promoter region of a gene to activate or repress transcription. This type of regulatory system is responsible for controlling many different functions in bacteria including virulence.<sup>62</sup> Two component regulatory systems have been identified in numerous bacteria and are involved in regulation of iron, phosphate, nitrogen, carbon, capsule production, and flagellar activity, to name a few.<sup>62</sup> The sensor molecule of the two component system generally contains a histidine kinase that autophosphorylates upon interaction with a signal molecule. The phosphate derived from ATP on the kinase is then transferred to the response regulator inducing a conformational change that results in binding or release of promoter DNA. The response

regulator may interact with RNA polymerase to increase transcription or it may bind a promoter region to prevent transcription of the gene. Bacterial virulence factors known to be regulated by two component systems include pertussis toxin of *B pertussis* (BvgA/BvgS<sup>63</sup>), pili formation and cholera toxin production of *V cholerae* (ToxR/ToxS<sup>64</sup>), *Salmonella* survival in macrophages (PhoP/PhoQ<sup>65</sup>), outer membrane porin regulation in *Salmonella* and *E coli*,<sup>66</sup> alginate production in *P aeruginosa* (FimS [AlgZ]/AlgR<sup>67</sup>), Yops proteins of *Yersinia pestis*, and iron regulation in *Salmonella* and *Pseudomonas* (Fur).<sup>68–70</sup> The extracellular signal that the bacterium is sensing for many of these two component systems is unknown. The number of similarities among these different two component systems may lead to the discovery of a novel therapeutic agent that could inhibit bacterial signal transduction processes.

Bacterial pathogens use common regulatory mechanisms, such as alternative sigma factors and two component signal transduction systems, to control the expression of their virulence genes in response to environmental conditions encountered during infection of the human host, including changes in temperature, pH, osmotic strength, oxygen availability, and nutrient conditions.

## EVOLUTION OF BACTERIAL PATHOGENS

The genetic makeup of bacterial genomes is subject to rapid and dramatic change through a variety of processes collectively referred to as "horizontal gene transfer". Recent evidence has shown that horizontal gene transfer plays a principal part in the molecular evolution of novel bacterial pathogens.<sup>71–73</sup> Horizontal gene transfer refers to the incorporation of genetic elements transferred from a donor organism directly into the genome of the recipient organism, where they form genomic islands—that is, blocks of DNA which contain mobile genetic elements. Genomic islands may contain large blocks of virulence determinants (adhesins, invasins, toxins, protein secretion systems, antibiotic resistance mechanisms, etc), and thus are referred to as pathogenicity islands. Pathogenicity islands were first described in pathogenic species of *E coli*, but have since been found in the genomes of numerous bacterial pathogens of humans, animals, and plants (*Salmonella*, *Vibrio*, *Shigella*, *Yersinia*, *Listeria*, *S aureus*, etc).<sup>1, 74</sup> Pathogenicity islands consist of large regions of genomic DNA (approximately 10–200 kilobases) that are present in pathogenic bacterial strains but absent from the genomes of non-pathogenic members of the same or related species. Pathogenicity islands are believed to have been acquired as a block by horizontal gene transfer because (a) their G+C content is significantly different from that of the genomes of the host micro-organism; (b) they are often flanked by direct repeats; (c) they are often associated with tRNA genes; (d) they are associated with integrase determinants and other mobility loci; and (e) they exhibit genetic instability (reviewed in Hacker and Kaper<sup>75</sup>). It is important to note that, in addition to pathogenicity islands, plasmids and bacteriophages can also be transferred horizontally. Indeed, all three mechanisms for genetic exchange or transfer between bacteria (that is, transformation, transduction, and conjugation) appear to be important for the evolution of pathogenic species. The determination and analysis of the complete genomic sequences of several important bacterial pathogens has led to the revelation that horizontal gene transfer may be much more extensive than previously appreciated.<sup>72</sup> Accordingly, a more comprehensive understanding of the evolution of bacterial pathogens will be required to elucidate the virulence mechanisms of emerging and re-emerging infectious diseases, as well as changes in virulence and drug resistance associated

with these infections, so that effective diagnostic and therapeutic strategies may be developed.

### ANTIBIOTIC RESISTANCE

The discovery of antibiotics over 50 years ago revolutionised medical treatment of infectious bacterial diseases. However, the widespread use of antibiotics over the past several decades has led to the emergence of antibiotic resistant strains of many bacteria, and represents a serious global threat to modern medical practice. Both Gram negative and Gram positive bacteria have acquired resistance to antimicrobial drugs. Antibiotic resistant bacterial strains (many of which have acquired multidrug resistance) that have recently emerged and are a cause for significant concern include: diarrhoeal pathogens such as *Shigella*, *Salmonella*, *E coli*, and *Enterococcus faecium*; respiratory pathogens like *Klebsiella pneumoniae* and *P aeruginosa*; urinary tract pathogens like *E coli*, and *M tuberculosis* which remains the leading cause of death from a single infectious disease worldwide.<sup>76-78</sup> Moreover, methicillin resistant *S aureus*, one of the most common causative agents of nosocomial infections, and vancomycin resistance in Gram positive organisms such as *Enterococcus* spp and *S aureus* are presenting significant challenges to modern clinicians in the effective treatment and management of infectious diseases.<sup>78-80</sup> An important question then becomes: Does exposure to antibiotics induce resistance? Evidence indicates that in *Bacteroides* a 100-fold increase in gene transfer has been observed in bacteria harboring conjugative transposons (all encoding tetracycline resistance) on exposure to low concentrations of tetracycline. In addition, subinhibitory concentrations of tetracycline induce resistance to this antibiotic in strains of *S pneumoniae* containing the *tet(M)* gene.<sup>81 82</sup>

There are three common types of antimicrobial resistance mechanisms in bacteria: those that modify the target site, those that alter uptake of the antibiotic, and those that inactivate the antibiotic. The acquisition of antibiotic resistance occurs by two genetic processes: by spontaneous mutations and mainly by the acquisition of genes from exogenous sources via horizontal transfer (see "Evolution of bacterial pathogens" above). Horizontal gene transfer occurs as genetic elements are transferred from one organism to another, intraspecies and interspecies. These genetic elements may be transferred as mobile elements such as transposons, by the uptake of naked DNA through transformation, by sexual transfer through conjugation, or by incorporation of DNA into a phage genome. For example, pathogenicity islands (see "Evolution of bacterial pathogens" above) are segments of DNA which carry factors which facilitate the survival of an organism under stressful conditions. The acquisition of a pathogenicity island containing an antibiotic resistance gene by one of the processes described above could result in acquired antibiotic resistance by an organism. Likewise, it has recently been shown that multidrug resistance of *S typhimurium* DT104, a food borne pathogen responsible for human enteritis, is due to the integration of a transposon carrying a resistance cassette into the strain's genome.<sup>77</sup> In addition, genetic change that leads to antibiotic resistance can be the result of a spontaneous mutation, a change of the genetic code. For example, a mutation which alters the binding site of a drug would decrease antibiotic sensitivity and thus increase drug resistance. Specifically, *M tuberculosis*, the causative agent of tuberculosis, remains a significant global health threat as it has acquired multiple drug resistance, including resistance to isoniazid and streptomycin, the latter of which occurred as a result of mutations altering the target site of the antibiotic.<sup>81</sup>

The ability of antibiotic resistant strains of bacteria to spread is a real danger that threatens worldwide public health. This process may be facilitated by the formation of biofilms which are organised communities of microbes that allow socialisation and shared living and can increase resistance to

environmental stresses. Spread can occur from animal to animal by contamination of food source with manure, from animal to human by ingestion of contaminated food, from the import or export of live animals or products, and from human to human, especially in healthcare settings.

The threat of an antibiotic resistant bacterial epidemic and/or pandemic is real and presents a formidable challenge to the global treatment of infectious diseases. Accordingly, innovations in drug research and infection control measures in the healthcare setting must be implemented to control and prevent the spread of these microbes. Recent progress in the quest for identification of factors contributing to bacterial virulence has led to the development of techniques such as in vivo expression technology,<sup>83</sup> differential fluorescence induction,<sup>84</sup> and signature transposon mutagenesis,<sup>85</sup> that provide valuable information on the infection process at the molecular level of the pathogen. In addition, recent advances in bioinformatics have resulted in the complete sequencing of the genomes of several bacterial pathogens (<http://www.tigr.org>), as well as structural, functional, and comparative analyses between these genomes. The potential use of genomics based information to identify targets in pathogenic bacteria may ultimately lead to the design of novel therapeutic treatments, which are so desperately needed to replace current antibiotic regimens.

The rapid emergence of bacterial pathogens with novel virulence and resistance properties is largely attributed to the process of horizontal transfer.

### INTERACTION OF PATHOGENS WITH THE INNATE IMMUNE SYSTEM

Micro-organisms are exposed to a barrage of non-specific barriers to infection after introduction of the microbe into the host. These barriers are part of the innate immune system and include epithelial cells of the skin, upper respiratory tract and genitourinary tract, antimicrobial substances in secretions, such as lysozyme in tears, low pH of stomach acid, complement proteins in the blood, and leucocytes in the blood and tissues. One criterion for a micro-organism to be pathogenic to man is that the microbe has the ability to survive these innate immunity insults and proliferate in the host. Once a pathogenic micro-organism has been introduced into the host, there is a "race" between the pathogen and the host to gain the upper hand in establishing infection by the pathogen or eliminating the pathogen by the host.

A critical first step in effective removal of a pathogenic microbe by the innate immune system depends on recognition of the microbe. The innate immune system has taken advantage of the existence of certain molecular patterns exhibited by pathogenic micro-organisms to use for recognising the microbe as potentially "dangerous" to the host. Pathogen associated molecular patterns, or PAMPs, are fairly invariant molecules made by the pathogen but not the host, and are usually required by the pathogen for survival or pathogenicity (reviewed in Medzhitov and Janeway<sup>86</sup>). PAMPs include LPS, peptidoglycan, lipoteichoic acids, certain bacterial DNA sequences containing CpG motifs, and certain polysaccharides. PAMPs on the pathogens are recognised by pattern recognition receptors on host cells. However, bacteria have evolved surface molecules involved in adherence to host tissues (see "Adherence" section). These adherence molecules facilitate uptake into the host that in some cases minimises host reactivity to the bacterium. If the host is able to bind to the invading bacterium via a pattern recognition receptor, the host is triggered to respond by enhanced endocytosis/phagocytosis and killing and or signalling to the nucleus to induce transcription of immunomodulatory factors.



Pattern recognition receptors can be grouped into three categories: secreted, endocytic, and signalling.<sup>86</sup> Secreted pattern recognition receptors include the surfactant proteins such as surfactant protein A that aids in the clearance of lung pathogens<sup>87</sup> and mannan binding lectin, which is found in the blood. Mannan binding lectin is a calcium dependent lectin that binds carbohydrate moieties on bacterial surfaces. Binding of mannan binding lectin induces a cascade of proteolytic cleavages of complement proteins leading to the deposition of pore forming complement proteins in the bacterial membrane and ultimately death of the bacterium.<sup>88</sup> Pathogenic micro-organisms have evolved numerous mechanisms to inhibit complement deposition via the mannan binding lectin pathway, as well as the alternative and classical pathways of complement activation. These complement evasion/inhibition mechanisms enhance the ability of the pathogen to survive and replicate in the host.

Examples of endocytic pattern recognition receptors are the mannose receptor, galactose receptor, and macrophage scavenger receptors. The mannose receptor is another C-type lectin that binds molecules containing mannoses and other polysaccharides and aids in the endocytosis of bacteria.<sup>89</sup> The lipoarabinomannan of *M. tuberculosis* is recognised by the mannose receptor when a terminal mannose is exposed.<sup>90</sup> Macrophage scavenger receptors bind to a variety of molecules including phospholipids, lipoproteins, and other negatively charged molecules and enhance endocytosis of bacteria (reviewed in Kreiger and Herz<sup>91</sup>).

The signalling group of pattern recognition receptors include the family of Toll-like receptors (reviewed in Aderem and Ulevitch<sup>92</sup>). Toll-like receptors have been shown to induce signals in host cells following binding of LPS, lipoproteins, peptidoglycan, lipoteichoic acid, lipoarabinomannan, and CpG-containing DNA.<sup>92-93</sup> In most cases, signalling via the Toll-like receptors results in the production of proinflammatory cytokines by host cells that aids in clearance of the pathogenic micro-organism. Currently, the number of Toll-like receptors in humans has risen to 10 with certain ones exhibiting restricted expression on tissues and cells.<sup>94</sup>

Pathogenic bacteria have evolved exquisite mechanisms for colonising humans and replicating in the host. Likewise, the host has developed innate immune mechanisms, which allow recognition of these invading organisms and discrimination between the host and the pathogen. Thus, bacteria face a formidable and potentially hostile environment when introduced into a host and they must overcome the insults thrown at them by the host in order to initiate and maintain a productive infection.

The pattern recognition receptors of the innate immune system are exquisitely designed to recognise conserved molecules (PAMPs) of pathogenic bacteria. Often the bacteria and the innate immune system of the host are in a "race" to either establish infection in the host or eliminate the bacteria from the host.

## FUTURE PERSPECTIVES

What does the future hold for the study of the mechanisms of microbial pathogenesis? Recent advances in high throughput polymerase chain reaction and DNA sequencing techniques, and microarray based gene expression profiling have allowed scientists to rapidly determine the complete genomic sequences of both microbial pathogens and eukaryotic hosts, as well as to measure levels of gene expression and provide a molecular description of the events that follow infection. The application of these methods to the genomes of microbial pathogens and their eukaryotic hosts, combined with efficient

analytical tools and genome scale approaches for studying gene expression, is revolutionising the development of novel tools for diagnosis, prognosis, and clinical management of infectious disease. Indeed, the use of these technologies has led to the discovery of shared secretory machineries, common regulatory mechanisms, and homology among secreted virulence effector molecules. These findings collectively led to the discovery that bacterial pathogenicity genes are commonly found as discrete islands in the chromosomal and extrachromosomal elements of pathogenic species, but are not found in non-pathogenic members of the same genus or species. Moreover, the recent application of high density genomic profiling to examine the molecular response of host tissues after infection with a number of microbial pathogens has begun to elucidate the complex interactions which occur between the host and pathogen during the infection process.<sup>95-96</sup>

The availability of numerous complete genome sequences of bacterial pathogens has significantly contributed to our understanding of the infectious disease process and has led to the realisation that many of these organisms use common mechanisms to cause infection and disease.

We anticipate that new methods and approaches will be developed to advance the rate of our elucidation of microbial pathogenesis. It is important to bear in mind that, although significant advances have been made in understanding the dynamic interactions that occur between the pathogen and the host during the infection process, our knowledge of these processes is still in its infancy. As our fundamental knowledge of the common themes in microbial pathogenicity increases, it can be anticipated that the means by which microbial infection can be controlled by the use of vaccines and other novel therapeutic approaches will lessen the likelihood and therefore the consequences of infectious diseases. Such advances will provide desperately needed innovative treatments for the increasing prevalence of deadly infectious diseases which have acquired multiple resistance to antibiotics.

## Questions (answers at end of paper)

1. What is the role of the bacterial capsule in evasion of the immune response?
2. Which components of the bacterial cell wall are the main causes of the induction of cytokines which lead to septic shock?
3. What are the differences between fimbrial and afimbrial adhesins?
4. How do cells of the innate immune system recognise potentially pathogenic bacteria?
5. What are the three intracellular niches that are occupied by bacteria which can survive and replicate within host cells?
6. What is the role of horizontal gene transfer in the evolution of bacterial pathogenesis?

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## REFERENCES

- 1 Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited. *Micro Mol Biol Rev* 1997;**61**:136-69.

- 2 **Garcia E**, Lull D, Lopez R. Functional organization of the gene cluster involved in the synthesis of the pneumococcal capsule. *Int Microbiol* 1999;**2**:169–76.
- 3 **Feldman C**, Klugman KP. Pneumococcal infections. *Curr Opin Infect Dis* 1997;**10**:109–15.
- 4 **Liu TY**, Gotschlich EC, Jonssen EK, *et al*. Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharide. *J Biol Chem* 1971;**246**:2849–58.
- 5 **Bhattacharjee AK**, Jennings JH, Kenny CP, *et al*. Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and B01. *Can J Biochem* 1976;**54**:1–8.
- 6 **Govan JR**, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 1996;**60**:539–74.
- 7 **Periti P**. Current treatment of sepsis and endotoxaemia. *Expert Opin Pharmacother* 2000;**1**:1203–17.
- 8 **Horn D**, Morrison D, Opal S, *et al*. What are the microbial components implicated in the pathogenesis of sepsis? *Clin Infect Dis* 2000;**31**:851–8.
- 9 **Verhoef J**, Mattsson E. The role of cytokines in gram-positive bacterial shock. *Trends Microbiol* 1995;**3**:136–40.
- 10 **Wright S**, Ramos R, Tobias P, *et al*. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990;**249**:1431–3.
- 11 **Das U**. Critical advances in septicemia and septic shock. *Crit Care* 2000;**4**:290–6.
- 12 **Guha M**, Mackman N. LPS induction of gene expression in human monocytes. *Cell Signal* 2001;**13**:85–94.
- 13 **Walker T**. *Microbiology*. Philadelphia: WB Saunders Company; 1998.
- 14 **Hamood A**, Wick M, Iglewski B. Secretion of toxin A from *Pseudomonas aeruginosa* PAO1, PAK, and PA103 by *Escherichia coli*. *Infect Immun* 1990;**58**:1133–40.
- 15 **Nakao H**, Takeda T. *Escherichia coli* Shiga toxin. *J Nat Toxins* 2000;**9**:299–313.
- 16 **Klose KE**. Regulation of virulence in *Vibrio cholerae*. *Int J Med Microbiol* 2001;**291**:81–8.
- 17 **Fujii G**, Choe SH, Bennett MJ, *et al*. Crystallization of diphtheria toxin. *J Mol Biol* 1991;**222**:861–4.
- 18 **Stein PE**, Boodhoo A, Armstrong GD, *et al*. Structure of a pertussis toxin-sugar complex as a model for receptor binding. *Nat Struct Biol* 1994;**1**:591–6.
- 19 **Merritt EA**, Hol WG. AB5 toxins. *Curr Opin Struct Biol* 1995;**5**:165–71.
- 20 **Domenighini M**, Magagnoli C, Pizzi M, *et al*. Common features of the NAD-binding and catalytic site of ADP-ribosylating toxins. *Mol Microbiol* 1994;**14**:41–50.
- 21 **Schiavo G**, Benfenati F, Poulain B, *et al*. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* 1992;**359**:832–5.
- 22 **Toder DS**, Gambello MJ, Iglewski BH. *Pseudomonas aeruginosa* LasA: a second elastase under the transcriptional control of lasR. *Mol Microbiol* 1991;**5**:2003–10.
- 23 **Engel LS**, Hill JM, Caballero AR, *et al*. Protease IV, a unique extracellular protease and virulence factor from *Pseudomonas aeruginosa*. *J Biol Chem* 1998;**273**:16792–7.
- 24 **Welch RA**. Pore-forming cytotoxins of gram-negative bacteria. *Mol Microbiol* 1991;**5**:521–8.
- 25 **Andrews NW**, Portnoy DA. Cytolysins from intracellular pathogens. *Trends Microbiol* 1994;**2**:261–3.
- 26 **Klauser T**, Pohlner J, Meyer TF. The secretion pathway of IgA protease-type proteins in gram-negative bacteria. *Bioessays* 1993;**15**:799–805.
- 27 **Savarino SJ**, Fasano A, Watson J, *et al*. Enterotoxigenic *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of E coli heat-stable toxin. *Proc Natl Acad Sci U S A* 1993;**90**:3093–7.
- 28 **Wilkins TD**, Iyer DM. Clostridium difficile toxins attack Rho. *Trends Microbiol* 1996;**4**:49–51.
- 29 **Aktories K**, Schmidt G, Just I. Rho GTPases as targets of bacterial protein toxins. *Biol Chem* 2000;**381**:421–6.
- 30 **Falzano L**, Fiorentini C, Boquet P, *et al*. Interaction of *Escherichia coli* cytotoxic necrotizing factor type 1 (CNF1) with cultured cells. *Cytotechnology* 1993;**11** (suppl 1):S56–8.
- 31 **Oswald E**, Sugai M, Labigne A, *et al*. Cytotoxic necrotizing factor type 2 produced by virulent *Escherichia coli* modifies the small GTP-binding proteins Rho involved in assembly of actin stress fibers. *Proc Natl Acad Sci U S A* 1994;**91**:3814–8.
- 32 **Donnenberg MS**. Pathogenic strategies of enteric bacteria. *Nature* 2000;**406**:768–74.
- 33 **Merz A**, So M. Interactions of pathogenic neisseriae with epithelial cell membranes. *Annu Rev Cell Dev Biol* 2000;**16**:423–57.
- 34 **Hahn H**. The type-4 pilus is the major virulence-associated adhesin of *Pseudomonas aeruginosa*—a review. *Gene* 1997;**192**:99–108.
- 35 **Joh D**, Wann E, Kreikemeyer B, *et al*. Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. *Matrix Biol* 1999;**18**:211–23.
- 36 **Novak R**, Tuomanen E. Pathogenesis of pneumococcal pneumonia. *Semin Respir Infect* 1999;**14**:209–17.
- 37 **Cleary P**, Cue D. High frequency invasion of mammalian cells by  $\beta$  hemolytic streptococci. *Subcell Biochem* 2000;**33**:137–66.
- 38 **Bermudez L**, Sangari F. Mycobacterial invasion of epithelial cells. *Subcell Biochem* 2000;**33**:231–49.
- 39 **Daffe M**, Etienne G. The capsule of *Mycobacterium tuberculosis* and its implications for pathogenicity. *Tuber Lung Dis* 1999;**79**:153–69.
- 40 **Zhang J**, Mostov K, Lamm M, *et al*. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. *Cell* 2000;**102**:827–37.
- 41 **Kenny B**, DeVinney R, Stein M, *et al*. Enteropathogenic *E coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 1997;**91**:511–20.
- 42 **Flock J**. Extracellular-matrix-binding proteins as targets for the prevention of *Staphylococcus aureus* infections. *Mol Med Today* 1999;**5**:532–7.
- 43 **Kelly C**, Younson J. Anti-adhesive strategies in the prevention of infectious disease at mucosal surfaces. *Expert Opin Investig Drugs* 2000;**9**:1711–21.
- 44 **Dziewanowska K**, Patti J, Deobald C, *et al*. Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. *Infect Immun* 1999;**67**:4673.
- 45 **Fleiszig SM**, Wiener-Kronish J, Miyazaki H, *et al*. *Pseudomonas aeruginosa*-mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exoenzyme. *Infect Immun* 1997;**65**:579–86.
- 46 **Dehio C**, Gray-Owen S, Meyer T. Host cell invasion by pathogenic *Neisseriae*. *Subcell Biochem* 2000;**33**:61–96.
- 47 **Sansonetti P**, Tran Van Nhieu G, Egile C. Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin Infect Dis* 1999;**28**:466–75.
- 48 **Galan JE**, Zhou D. Striking a balance: modulation of the actin cytoskeleton by salmonella. *Proc Natl Acad Sci U S A* 2000;**97**:8754–61.
- 49 **Hackstadt T**. Redirection of host vesicle trafficking pathways by intracellular parasites. *Traffic* 2000;**1**:93–9.
- 50 **Russell D**. Where to stay inside the cell: a homesteader's guide to intracellular parasitism. In: Cossart P, Boquet P, Normark S, *et al*, eds. *Cellular microbiology*. Washington, DC: ASM Press, 2000.
- 51 **Chiang S**, Mekalanos J, Holden D. In vivo genetic analysis of bacterial virulence. *Annu Rev Microbiol* 1999;**53**:129–54.
- 52 **Graham JE**, Clark-Curtiss JE. Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). *Proc Natl Acad Sci U S A* 1999;**96**:11554–9.
- 53 **Loewen P**, Hengge-Aronis R. The role of the sigma factor sigma S (KatF) in bacterial global regulation. *Annu Rev Microbiol* 1994;**48**:53–80.
- 54 **Fang FC**, Libby SJ, Buchmeier NA, *et al*. The alternative sigma factor KatF (RpoS) regulates *Salmonella* virulence. *Proc Natl Acad Sci U S A* 1992;**89**:11978–82.
- 55 **Wang Y**, Kim K. Effect of rpoS mutations on stress-resistance and invasion of brain microvascular endothelial cells in *Escherichia coli* K1. *FEMS Microbiol Lett* 2000;**182**:241–7.
- 56 **Suh S**, Silo-Suh L, Woods D, *et al*. Effect of rpoS mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa*. *J Bacteriol* 1999;**181**:3890–7.
- 57 **Bachman M**, Swanson M. RpoS co-operates with other factors to induce *Legionella pneumophila* virulence in the stationary phase. *Mol Microbiol* 2001;**40**:1201–14.
- 58 **Humphreys S**, Stevenson A, Bacon A, *et al*. The alternative sigma factor, sigmaE, is critically important for the virulence of *Salmonella typhimurium*. *Infect Immun* 1999;**67**:1560–8.
- 59 **Schurr M**, Yu H, Boucher J, *et al*. Multiple promoters and induction by heat shock of the gene encoding the alternative sigma factor AlgU (sigma E) which controls mucoidy in cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J Bacteriol* 1995;**177**:5670–9.
- 60 **Parsot C**, Mekalanos J. Expression of ToxR, the transcriptional activator of the virulence factors in *Vibrio cholerae*, is modulated by the heat shock response. *Proc Natl Acad Sci U S A* 1990;**87**:9898–902.
- 61 **Akerley B**, Miller J. Flagellin gene transcription in *Bordetella bronchiseptica* is regulated by the BvgAS virulence control system. *J Bacteriol* 1993;**175**:3468–79.
- 62 **Hoch JA**, Silhavy TJ. *Two-component signal transduction*. Washington, DC: ASM Press, 1995.
- 63 **Uhl MA**, Miller JF. Integration of multiple domains in a two-component sensor protein: the *Bordetella pertussis* BvgAS phosphorelay. *EMBO J* 1996;**15**:1028–36.
- 64 **DiRita VJ**. Co-ordinate expression of virulence genes by ToxR in *Vibrio cholerae*. *Mol Microbiol* 1992;**6**:451–8.
- 65 **Groisman E**. The pleiotropic two-component regulatory system PhoP-PhoQ. *J Bacteriol* 2001;**183**:1835–42.
- 66 **Forst SA**, Roberts DL. Signal transduction by the EnvZ-OmpR phosphotransfer system in bacteria. *Res Microbiol* 1994;**145**:363–73.
- 67 **Deretic V**, Dikshit R, Konyecsni WM, *et al*. The algR gene, which regulates mucoidy in *Pseudomonas aeruginosa*, belongs to a class of environmentally responsive genes. *J Bacteriol* 1989;**171**:1278–83.
- 68 **Hoe NP**, Goguen JD. Temperature sensing in *Yersinia pestis*: translation of the LcrF activator protein is thermally regulated. *J Bacteriol* 1993;**175**:7901–9.
- 69 **Skurnik M**, Toivanen P. LcrF is the temperature-regulated activator of the yadA gene of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *J Bacteriol* 1992;**174**:2047–51.
- 70 **Vasil ML**, Ochsen UA. The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. *Mol Microbiol* 1999;**34**:399–413.
- 71 **Ochman H**, Moran N. Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. *Science* 2001;**292**:1096–9.
- 72 **Wren B**. Microbial genome analysis: insights into virulence, host adaptation and evolution. *Nat Rev Genet* 2000;**1**:30–9.
- 73 **Ziebuhr W**, Ohlsen K, Karch H, *et al*. Evolution of bacterial pathogenesis. *Cell Mol Life Sci* 1999;**56**:719–28.
- 74 **Lindsay J**, Ruzin A, Ross H, *et al*. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. *Mol Microbiol* 1998;**29**:527–43.



- 75 **Hacker J**, Kaper J. Pathogenicity islands and the evolution of microbes. *Annu Rev Microbiol* 2000;**54**:641–79.
- 76 **Mazel D**, Davies J. Antibiotic resistance in microbes. *Cell Mol Life Sci* 1999;**56**:742–54.
- 77 **Teuber M**. Spread of antibiotic resistance with food-borne pathogens. *Cell Mol Life Sci* 1999;**56**:755–63.
- 78 **Gold H**, Moellering R. Antimicrobial drug resistance. *Drug Ther* 1996;**335**:1445–53.
- 79 **Froloff G**. Why is transmission of vancomycin-resistant enterococci on the increase? *Prog Transplant* 2001;**11**:17–22.
- 80 **Tenover F**, Biddle J, Lancaster M. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. *Emerg Infect Dis* 2001;**7**:327–32.
- 81 **Davies J**. Inactivation of antibiotics and the dissemination of resistance genes. *Science* 1994;**264**:375–82.
- 82 **Doherty N**, Trzcinski K, Pickerill P, et al. Genetic diversity of the tet(M) gene in tetracycline-resistant clonal lineages of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2000;**44**:2979–84.
- 83 **Mahan M**, Slauch J, Mekalanos J. Selection of bacterial virulence genes that are specifically induced in host tissues. *Science* 1993;**259**:686–8.
- 84 **Valdivia R**, Falkow S. Fluorescence-based isolation of bacterial genes expressed within host cells. *Science* 1997;**277**:2007–11.
- 85 **Hensel M**, Shea J, Gleeson C, et al. Simultaneous identification of bacterial virulence genes by negative selection. *Science* 1995;**269**:400–3.
- 86 **Medzhitov R**, Janeway C. Innate immunity. *N Engl J Med* 2000;**343**:338–44.
- 87 **Schagat T**, Tino M, Wright J. Regulation of protein phosphorylation and pathogen phagocytosis by surfactant protein A. *Infect Immun* 1999;**67**:4693–9.
- 88 **Fraser I**, Exekowitz R. Mannose receptor and phagocytosis. *Phagocytosis and pathogens*. Greenwich, CT: JAI Press, 1999: 85–99.
- 89 **Neth O**, Jack D, Dodds A, et al. Mannose-binding lectin binds to a range of clinically relevant organisms and promotes complement deposition. *Infect Immun* 2000;**68**:688–93.
- 90 **Kang B**, Schlesinger L. Characterization of mannose receptor-dependent phagocytosis mediated by *Mycobacterium tuberculosis* lipoarabinomannan. *Infect Immun* 1998;**66**:2769–77.
- 91 **Kreiger M**, Herz J. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu Rev Biochem* 1994;**63**:601–37.
- 92 **Aderem A**, Ulevitch R. Toll-like receptors in the induction of the innate immune response. *Nature* 2000;**406**:782–7.
- 93 **Hacker H**, Vabulas R, Takeuchi O, et al. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF) 6. *J Exp Med* 2000;**192**:595–600.
- 94 **Chuang T**, Ulevitch R. Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. *Biochim Biophys Acta* 2001;**1518**:157–61.
- 95 **Eckmann L**, Smith JR, Housley MP, et al. Analysis by high density cDNA arrays of altered gene expression in human intestinal epithelial cells in response to infection with the invasive enteric bacteria *Salmonella*. *J Biol Chem* 2000;**275**:1408–94.
- 96 **Detweiler CS**, Cunanan DB, Falkow S. Host microarray analysis reveals a role for the *Salmonella* response regulator phoP in human macrophage death. *Proc Natl Acad USA* 2000;**98**:5850–5.

## ANSWERS

1. The presence of a capsule on a bacterial surface can serve to minimise complement activation and prevent ingestion of bacteria by phagocytes. This inhibits clearance of the bacteria at the site of infection.

2. Bacterial LPS (also known as endotoxin) is found in the cell wall of Gram negative bacteria and is usually considered to be the principal component responsible for the induction of septic shock. In Gram positive bacteria, peptidoglycan fragments and teichoic acids found in the cell wall elicit many of the same physiological responses as LPS in the infected host.

3. Fimbrial adhesins are long, protruding, helical shaped structures formed from a single repeating protein subunit and other subunits at the tip and base. The fimbrial tip frequently serves to recognise a host receptor. Afimbrial adhesins are generally membrane associated proteins that do not extend as a fimbrial structure from the bacterial envelope. These adhesins are used for interactions with the host cell which occur over a shorter range than with fimbriae.

4. Cells of the innate immune system utilise germline encoded receptors that bind to conserved molecules on the bacteria called PAMPs. Binding of the PAMPs by pattern recognition receptors on the host cells can enhance endocytosis/phagocytosis and killing of the bacteria and/or induce signalling cascades in the host cells leading to proinflammatory cytokine and chemokine expression and secretion.

5. The phagolysosomal vacuole, the non-lysosomal, “specialised” vacuole, and the host cell cytosol.

6. Many bacterial pathogens encode virulence factors and antibiotic resistance determinants on unstable DNA regions, which can be readily transferred to bacteria of the same species or even to non-related prokaryotes via horizontal gene transfer. Since these mobile genetic elements often contain large blocks of virulence determinants, horizontal gene transfer is one of the main mechanisms in the evolution of bacterial pathogens, and is responsible for the rapid emergence of variants with novel virulence and resistance properties.