

# Frontal and stealth attack strategies in microbial pathogenesis

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**Interactions between microbes and human hosts can range from a benign, even symbiotic collaboration to a competition that may turn fatal — resulting in death of the host, the microbe or both. Despite advances that have been made over the past decades in understanding microbial pathogens, more people worldwide still die every year from infectious disease than from any other cause. This highlights the relevance of continuing to probe the mechanisms used by microorganisms to cause disease, and emphasizes the need for new model systems to advance our understanding of host–pathogen interactions.**

Although a wide range of microbe–host relationships can ultimately lead to disease, the two most general strategies used by pathogenic microbes may be described in military terms as 'frontal' and 'stealth' assaults. Pathogenic microorganisms make use of both of these approaches. Typically, frontal assault strategies require that the infecting microbes rapidly replicate, induce disease symptoms that overwhelm the innate defences of the host, and find a new host before engagement of the 'adaptive' or 'acquired' immune system, in which antigen-specific lymphocytes respond to antigen exposure. Stealth assaults, on the other hand, typically involve a slower infection process in which the microbes subvert the host's innate and adaptive immune systems to set up a chronic or persistent infection (Box 1). The diverse tactics used for both forms of attack have been the subject of intense investigation, which has helped not only to shape our understanding of the invading organisms, but also to define many of the pathways that are important in host resistance and susceptibility, and in normal host physiology. In discussing microbial pathogenicity, it is important to consider that every human is host to myriad microorganisms, and that illness is the exception rather than the rule (Box 2). Nevertheless, microbial pathogens impose a tremendous medical burden, so it is important that we understand their diverse assault strategies. Here we outline a variety of frontal and stealth strategies used by pathogenic microorganisms. In particular, we discuss the less well-known strategies used by chronically infecting bacteria, and outline the mechanisms used by these pathogens to subvert both the innate and the acquired immune systems.

## Frontal assault strategies

Many pathogenic microbes adopt an aggressive strategy in which they immediately attack and attempt to overwhelm the host's innate immune system. Although there are many variations to this mode of assault (Table 1), these microbes typically produce toxins or use complex secretion systems to deliver so-called effector proteins, which disrupt the normal function of the host cell.

One example of a pathogen that uses a frontal assault strategy is *Vibrio cholerae*, a potent epidemic adversary in many developing areas of the world. The organism is typically ingested in contaminated food and water, and produces a powerful toxin that causes a voluminous secretory diarrhoea[1]. In severely affected patients, the volume of stool purged can be in excess of the patient's own body weight[2] and dehydration is the typical cause of death. The ability of *V. cholerae* to rapidly cause disease before eliciting a productive immune response is shared by many microorganisms, including the most common cause of diarrhoeal disease in infants, the rotavirus family, which is responsible for more than 600,000 deaths a year[3].

Cholera toxin is encoded in the genome of a bacteriophage, called cholera-toxin phage, that is integrated into the *V. cholerae* genome. Thus, the virulence of *V. cholerae* results from the acquisition, by horizontal genetic transfer, of foreign DNA that encodes a toxin[4]. The receptor used by the cholera-toxin phage to infect *V. cholerae* is encoded as part of an additional portion of horizontally acquired DNA known as a pathogenicity island (PAI).

PAIs, found in many types of bacteria, are large chromosomal regions of DNA that are believed to have originated either from bacteriophages or from small, self-replicating bacterial DNA structures called plasmids[5]. This mechanism, in which acquired DNA has an important role in converting an otherwise harmless species into a pathogen, has been recognized as a common occurrence for many bacteria. It also serves as a microbial example of darwinian natural selection, in which the presence of pathogenic species is partially due to selective pressure applied by the host on the infecting microbe. For example, it is conceivable that before acquisition of the cholera toxin, the ancestral form of *V. cholerae* would colonize and reside in the small intestine but would cause few clinical symptoms. Eventually, this extended stay would result in engagement of the adaptive immune system. Because *V. cholerae* has few, if any, mechanisms to subvert the adaptive branch of the human immune system, most of the bacteria would be killed and not disseminated back to the environment. So although it is possible that the human was a 'dead end' for the ancestral form of *V. cholerae*, infection with the cholera-toxin phage produced a strain that could proliferate in the human host and be shed in great numbers back to the aquatic environment before productive engagement of the adaptive immune response. As a result, this pathogen has flourished. This illustrates how host diligence in the form of innate and adaptive immunity imposes selective pressures that shape pathogenic lifestyle, and helps to explain

Box 1
**General characteristics of frontal and stealth strategies of pathogenic bacteria**

Pathogenic bacteria use numerous strategies to overcome host defences. Broadly speaking, frontal and stealth assault strategies are distinguished by the following characteristics.

Frontal assault
- Short incubation period
- Acute clinical symptoms
- Engages the innate immune system
- Requires transmission to new susceptible hosts to maintain infection
- Rapid microbial replication
- Carrier state is uncommon
- Transmission often requires intimate contact
- Reservoir is often a specific host or the pathogens are opportunistic
- Often induces sterilizing immunity

Stealth assault
- Incubation period may be indeterminate
- Indolent or asymptomatic carriage
- Engages the innate immune system
- Avoids or manipulates the adaptive immune system
- Rapid microbial replication may punctuate a recurrent or terminal event
- Carrier state is common with periods of shedding
- Transmission is by direct contact or persistence in the environment
- Reservoir is usually a specific host or a group of related host species
- Rarely induces sterilizing immunity

why closely related pathogens have such different lifestyles and cause such diverse diseases.

Many bacterial pathogens have been shown to cause disease by delivering proteins directly into the host cell that they are infecting. This is done using specialized type III/IV secretion systems that are typically acquired as part of a PAI. This island encodes components that assemble into a 'molecular syringe', which can inject effector molecules into the host cell[6,7]. For example, the genus *Yersinia* contains three pathogenic species that use type III secretion to subvert the host immune response and cause diseases ranging from gastrointestinal disorders to bubonic plague. When it enters the host, *Yersinia* encounters macrophages and other specialized phagocytic cells, which serve as the first line of immunological defence. Interaction with these host cells causes *Yersinia* to produce and deliver proteins called *Yersinia* outer-membrane proteins (Yops), which prevent phagocytosis, disrupt normal signal transduction pathways and initiate the ultimate apoptotic demise of the phagocytic cells[6]. Unlike *V. cholerae*, which delivers its potent toxin and is then rapidly shed from the body in the diarrhoeal output, thereby avoiding a strong inflammatory response, *Yersinia* prefers a 'stand and fight' approach in which it faces and overcomes the host's innate and adaptive immune systems.

## Stealth assault strategies

Our understanding of the strategies used by pathogens to set up persistent infections remains fairly limited compared with our knowledge of the frontal assault strategies discussed above. It is important to point out that a high proportion of persistent infections are caused by viral and parasitic pathogens[8–11]. Nevertheless, chronically infecting bacteria present a significant challenge in terms of their ability to cause disease. The remainder of this review will focus primarily on microbes that use stealth assault strategies to cause chronic infections, and on recent findings that may further our understanding of the complex interplay between host and pathogen.

### *Helicobacter pylori*

Infecting over 50% of the world's population, *Helicobacter pylori* has been recognized as something of a bacterial role model for its ability to colonize and persist for the lifetime of the host, even when faced with a robust host immune system. The bacterium exclusively infects human and non-human primates, and persists in the harsh environment of the stomach. About 20% of those infected with *H. pylori* will ultimately suffer diseases ranging from gastritis and ulcer disease to gastric cancer[12]. This microbe, which uses a variety of strategies and gene products to subvert the innate and adaptive immune systems of the host (Fig. 1), therefore represents a tremendous medical challenge.

Gastric acidity is a major antimicrobial defence, and the stomach serves as a death chamber for most of the millions of bacteria that are ingested daily. However, *H. pylori* not only survives the low pH, but thrives in the human stomach. The bacterium has a number of strategies to overcome acid stress[13]. Foremost among these approaches is production of the bacterial enzyme urease, which catalyses the hydrolysis of urea to carbon dioxide and ammonia, and helps to maintain a protonmotive force, essential for bacterial metabolism and survival, across the *H. pylori* inner membrane[14,15]. The production of the basic ammonia molecule helps to buffer the bacterial cytoplasm and the microenvironment as it is secreted from the bacterial cell.

Another important part of the human innate immune system involves Toll-like receptors (TLRs). Bacterial lipopolysaccharide (LPS), a component of the the bacterial cell wall, is typically recognized by TLR4 and this triggers a robust proinflammatory response. However, recent work has shown that primary stomach epithelial cells and gastric epithelial cell lines do not react to the LPS from *H. pylori*[16]. In addition, it has been shown that recognition of *H. pylori* by TLR5 is markedly different from that observed with other Gram-negative pathogens, as *H. pylori* flagellins do not signal through TLR5 to stimulate an innate immune response[17,18]. Despite the lack of immunostimulation by these traditional pathways, *H. pylori* is reported to cause a strong inflammatory response *in vivo* through nuclear factor (NF)-κB activation. It has been suggested that this is required for establishment of a chronic infection[19]. So although the bacterium bypasses the innate TLR system, it can engage alternative inflammatory mediators.

*H. pylori* not only subverts the innate immune system, but also modulates the adaptive immune system by blocking the antigen-dependent proliferation of T cells. This is accomplished partially by delivery of vacuolating cytotoxin, VacA[20], which blocks T-cell receptor signalling events that normally lead to the production of cytokines, important mediators of the immune response.

A role in subverting the other branch of the adaptive immune response has been indicated by the finding that ectopic expression in B cells of the CagA protein — a product of the cytotoxin-associated gene (*cag*) PAI of *H. pylori* — inhibits B-cell proliferation by suppressing the JAK–STAT signalling pathway[21]. This study also suggested that CagA represses B-cell apoptosis, which may contribute to the formation of *H. pylori*-induced mucosa-associated lymphoid tissue (MALT) lymphoma. In this case, host immune defences that are designed to kill the invading microbe are subverted in such a way that they harm the host and not the bacterium.

As well as actively suppressing the immune response and the ability of the host to clear the infection, recent evidence suggests that *H. pylori* can hide from classic host defences by becoming intracellular.

Box 2
**Microbial pathogenicity as a survival strategy**

Of the 500 or so bacterial species that inhabit us, most never cause illness. But although these 'commensals' (literally 'to eat from the same table') are critical to human existence, some can cause disease in immunologically compromised hosts. This begs the question, what makes some of these flora able to cause disease?

Perhaps the best known of the commensal flora that cause life-threatening disease are those cultured from the human nasopharynx: *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* type B. Every human is, at one time or another, colonized by these species and most escape unharmed. Although the common assumption is that individuals who become ill do so when their carried strain becomes virulent, in fact the epidemiology of pneumococcal and meningococcal meningitis shows that disease is typically caused by newly acquired strains — even in individuals who had previously been carriers of the same species. The clinical reality is that most people harbour these organisms asymptomatically, and disease occurs infrequently in a minority of us.

Descriptions of virulence characteristics of these organisms generally focus on their ability to resist host defence mechanisms by synthesizing antiphagocytic substances, producing specific proteases that degrade host antibodies, and undergoing antigenic variation to avoid recognition by the immune system. However, this overlooks the fact that these 'pathogenicity' determinants probably evolved to facilitate persistent colonization and not disease. But why do certain organisms require these factors to colonize, when the vast majority of our microbial flora do not? One possible explanation is that they have a niche within the nasopharynx that involves crossing an anatomical barrier that keeps other flora at bay. We suggest that this barrier may be the nasopharyngeal-associated lymphatic tissue (NALT), and that these organisms persist in the small lymph nodes adjacent to the NALT in much the same way as *Salmonella* persists in lymph nodes adjacent to Peyer's patches. This would explain why immunization with the type B capsule of *H. influenzae* protects against both invasive disease and colonization.

This strategy for survival and persistence only pertains to host-adapted microbes. There are also opportunists, which depend on host defence calamity to cause disease. These microbes, usually acquired accidentally from animals, may kill us because we are an evolutionary dead end for them; we have not established the rules of engagement. However, in their host of choice, these same microbes usually behave like our host-adapted flora — with an eye towards persistence rather than towards causing disease and death. The balance shift that causes colonization to go awry, leading to a deadly disease, is a combination of physiological and genetic factors for both participants of the host–pathogen interaction.

In the end, pathogenicity is simply a strategy for microbial survival that involves the inherent ability to breach host barriers that stop other microorganisms. The need to replicate and acquire nutrients provides the driving force for this evolution, which enables the microbe to exploit a previously unoccupied niche. This incursion, and the acquisition of genes that make it possible, has in time become a necessity for survival of these microbes, as they have lost genes that were important for their pre-host-adapted life. The evolution of host–parasite adaptation is still with us, and will continue to be so as long as microorganisms outnumber us numerically and in their replicative power.

Although *H. pylori* is believed to remain primarily extracellular during infection, it was noted by early investigators examining gastric biopsies that a subpopulation of the bacteria could be found within cells[22,23]. The relevance of this observation was questioned for many years, but recent studies have shown that *H. pylori* can invade cultured eukaryotic cells and reside within multivesicular bodies that promote bacterial survival, motility and replication, and ultimately serve as a reservoir for re-establishment of infection (the intracellular bacteria egress from the vesicles and reseed the extracellular milieu)[24]. The relevance of these *in vitro* findings is supported by recent work showing that the bacterium could be detected inside precancerous and cancerous epithelial cells from *H. pylori*-positive patients[25]. In addition, the relative level of expression of several known bacterial virulence factors was considerably higher in the advanced-stage cancerous cells, suggesting that expression of virulence factors by these intracellular bacteria may play a part in disease progression. The ability of *H. pylori* to survive intracellularly and to escape many host immune defences is not unique. It is used by many microbial pathogens, including *Mycobacterium tuberculosis*, *Listeria monocytogenes* and *Shigella flexneri*, the causative agents of tuberculosis, listeriosis and shigellosis, respectively.

A final mechanism that may be crucial for *H. pylori* to escape the host immune response involves its ability to undergo genetic rearrangement that either eliminates particular immunostimulatory gene products or causes variation in potentially immunostimulatory molecules. Genomotyping of clonal isolates using an *H. pylori* microarray from a single human host suggests that as many as 3% of the loci show significant genetic variation[26]. Although the implications of this finding are not fully understood, a study showing that strains of *H. pylori* that have deleted all or portions of the PAI that delivers CagA to the host cell are more likely to colonize various animal models suggests that the ability of the bacterium to modulate gene products that affect the robustness of the immune response is significant[27].

Although CagA is a virulence factor for *H. pylori*, the presence of CagA is not sufficient to predict the disease outcome in *H. pylori* infection. It is known that individuals infected with CagA that can be phosphorylated have more severe disease than patients infected with a non-phosphorylateable CagA, but which of these forms of CagA was the progenitor in the evolution of *H. pylori* is unclear. A functional PAI is found in most human clinical isolates, but there is variation in the *cagA* coding region in the critical EPIYA domains that are phosphorylated by host tyrosine kinases[28].

These epidemiological results are supported by the recent finding that intragenomic recombination in the *cagA* coding sequence results in the production of a non-phosphorylateable form of CagA that does not induce morphological changes associated with pathogenicity in host cells[29]. The importance of this strategy of genetic rearrangement within the host may explain the recent identification of the RuvC protein, a Holliday junction resolvase involved in recombination, as a crucial factor in bacterial persistence[30].

Many other pathogens use genetic rearrangement as a means of immune escape. Included among these are *Neisseria gonorrhoeae* and HIV, which are responsible for gonorrhoea and AIDS, respectively.

### *Salmonella enterica* serovar Typhi

Many *Salmonella* serovars, like *Salmonella typhi*, the causative agent of typhoid fever, are adapted exclusively to human hosts and are endemic in areas of the world with poor sanitation and inadequate sewage-treatment facilities. Typhoid fever remains an important cause of human morbidity and mortality, and is becoming increasingly difficult to treat owing to the emergence of antibiotic-resistant strains[31]. Between 1% and 6% of those infected with *S. typhi* become chronic carriers of the bacterium and, although not necessarily suffering any symptoms themselves, may serve as a reservoir for subsequent infections during periods of faecal and urinal shedding[32] — think Typhoid Mary (see Box 3). Sites of persistence within the body have been shown to include the bone marrow and the gall bladder[33,34], but little

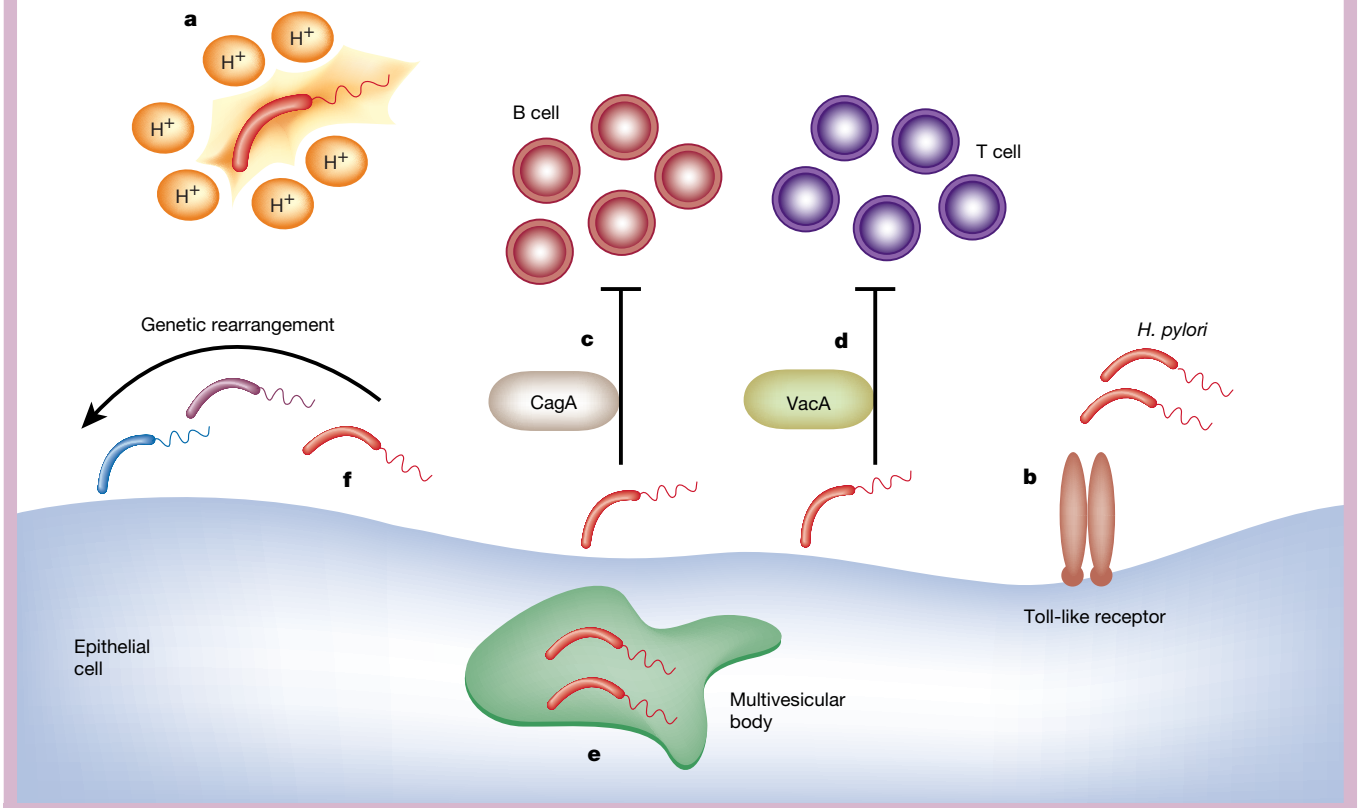


**Figure 1** Mechanisms used by *Helicobacter pylori* to establish persistent infection. The bacterium overcomes the innate immune system by neutralizing gastric acid (**a**) and by having lipopolysaccharide and flagella with low immunostimulatory recognition by Toll-like receptors (**b**). The adaptive immune system is also affected: B-cell (**c**) and T-cell (**d**) proliferation are blocked by the activity of CagA (a product of the cytotoxin-associated gene (*cag*) pathogenicity island) and VacA (vacuolating cytotoxin), respectively. In addition, the bacterium can escape exposure to host immune defences by surviving intracellularly (**e**), and shows a high frequency of genetic rearrangement (**f**) that appears to be essential for persistent colonization.

is understood about the adaptive mechanisms used by the bacteria during the course of establishing a persistent infection. Recent work using a novel model of bacterial persistence with *S. typhimurium* has begun to shed light on *Salmonella* persistence strategies[35] and should be amenable to genetic analysis, both to dissect the role of individual bacterial factors in persistence and to explain the failure of the host immune system to eradicate the infection.

Adaptation of *Salmonella* serovars to the host and the capacity of adapted microbes to persist are essential survival features of most, if not all, *Salmonella* serovars. Although *S. typhimurium* is well known as a cause of food-borne gastroenteritis in humans, this contributes little to the survival of the species because it rarely establishes a carrier state in humans and is associated only occasionally with intrafamilial spread. Mice infected with *S. typhimurium* have been used as a model to study acute systemic disease, as the bacterium causes a typhoid-like disease in some mouse strains. Differences in mouse susceptibility to *S. typhimurium* have been linked to the particular allele of the *Nramp1* gene being expressed in cells of the monocyte/macrophage lineage[36]. *Nramp1* is involved in controlling exponential growth of the bacteria in the reticuloendothelial system during the early stages of infection. In a recent study, mice expressing the wild-type *Nramp1* allele did not die after oral inoculation with *S. typhimurium*, but became uniformly persistently infected in a manner similar to that observed in chronic typhoid carriers[35]. In these animals, *Salmonella* was found to persist predominately in the mesenteric lymph nodes and/or spleen even in the face of a robust antibody response. Closer inspection showed that the bacteria were actually intracellular, having colonized macrophages. Most of the infected macrophages only contained between one and three bacteria — fewer than would be expected on the basis of studies in cultured macrophages, in which the SPI-2 pathogenicity island of *Salmonella* (a genetic element responsible for causing disease) is thought to permit intracellular replication. Whether SPI-2 is actually expressed in persistent infection of macrophages is not certain. However, reactivation of intracellular *Salmonella* and systemic spread could be accomplished by administering interferon-γ (IFN-γ)-neutralizing antibodies, suggesting that the host cytokine IFN-γ is important for suppression of *Salmonella* replication and disease[35]. IFN-γ is also important for the maintenance of latent infections of *Mycobacterium tuberculosis* and *Chlamydia trachomatis*. Using this persistence model and recent technological advances, it should be possible to identify bacterial factors that are important for persistence. This could be accomplished using microarrays and global screening strategies of libraries of defined *Salmonella* mutants[37].

### *Bartonella* spp.

The *Bartonella* genus is increasingly recognized as a significant human pathogen. *Bartonella* spp. cause numerous diseases, including Oroya fever, verruga peruana, trench fever, bacillary angiomatosis, endocarditis and cat-scratch disease[38]. *Bartonella* infections are disseminated from their natural reservoir (cats, rats, humans, deer and other mammals) by arthropods such as fleas and ticks. Carrier rates in the reservoir population are quite high: as many as 41% of domestic cats have been shown to be infected with *B. henselae*[39]. *Bartonellae* are fairly unique among bacterial pathogens in terms of their ability to sustain prolonged periods of parasitism within red blood cells. They have been shown to invade, multiply within and persist for the lifetime of the infected host cell[40], and to reach titres of $10^4$ per ml of blood in infected humans[41]. How this many Gram-negative organisms can persist within the bloodstream without inducing a classic

Table 1 **An overview of mechanisms used by bacteria to avoid host immune responses**

| Immune response affected | Pathogen | Bacterial factor/mechanism | References |
|---|---|---|---|
| Induction of apoptosis/cytotoxicity | *Shigella flexneri* | IpaB activation of pro-caspase 1 | 50 |
| | *Yersinia* | YopJ/P injection into macrophages | 6 |
| Inhibition of apoptosis | *Chlamydia* | IL-10-induced TNF-α suppression | 51 |
| | *Mycobacterium tuberculosis* | Increased expression of *bcl2* and Rb | 52 |
| Inhibition of cytokine production | *Bartonella* | IL-10 induction suppresses other cytokines | 43 |
| | *Vibrio cholerae* | CT inhibition of IL-12 secretion | 53 |
| Cytokine overproduction | *Bordetella pertussis* | Pertussis toxin induction of IL-1 and IL-4 | 54, 55 |
| | *Helicobacter pylori* | IL-8 induction | 12 |
| Complement evasion | *Streptococcus pyogenes* | M protein binding of C4BP | 56 |
| | *Porphyromonas gingivalis* | Protease inactivation of C3 and C5 | 57 |
| Evasion of host antibodies | *Staphylococcus aureus* | Protein A binding of IgG blocks phagocytosis | 58 |
| | *Peptostreptococcus magnus* | Protein L binding of κ light chains | 59 |
| Inhibition of antigen presentation | *Helicobacter pylori* | VacA targeting of antigen-presenting cells | 20 |
| | *Mycobacterium tuberculosis* | Downregulation of MHC class II and CD1 | 60 |
| Inhibition of phagocytosis | *Pseudomonas* | ExoT, ExoS targeting of Rho, Rac and Cdc42 | 61, 62 |
| | *Yersinia* | YopO targeting of RhoA, Rac and actin | 6 |
| Survival in phagocytes | *Legionella* | Dot/ICM genes inhibit phagolysosome fusion | 63, 64 |
| | *Coxiella burnetii* | Acid tolerance allows survival in acidified lysosomes | 65 |
| Phase/antigenic variation | *Neisseria gonorrhoeae* | Modulation of pilin | 66 |
| | *Helicobacter pylori* | Modulation of PAI and CagA | 27, 29 |

CagA, product of cytotoxin-associated gene (*cag*) PAI; CT, cholera toxin; ICM, intracellular multiplication; IgG, immunoglobulin-γ; IL, interleukin; MHC, major histocompatibility complex; PAI, pathogenicity island; TNF-α, tumour-necrosis factor-α; VacA, vacuolating cytotoxin.

septic shock response remains to be determined, but the finding suggests that, as with *H. pylori*, the LPS of *Bartonella* may have reduced immunostimulatory properties. Also, as for *Salmonella*, the ability to survive intracellularly helps *Bartonella* to escape the host immune response. Antibodies have no effect on *Bartonella* within red blood cells, but may contribute to preventing new waves of bacterial invasion[42]. It has also been suggested that intracellular *Bartonella* may affect the development of an adaptive immune response in a manner similar to that seen with malaria-infected red blood cells[43]. Malaria prevents the maturation of dendritic cells, which are essential for the development of a normal adaptive immune response[44].

Genetic studies and development of appropriate animal models of haemotropic infection by *Bartonella* have begun to shed light on some of the bacterial factors that are required for intraerythrocytic infection (Fig. 2). One important virulence factor in *Bartonella* is the type IV secretion system. It was recently shown that mutation of either *virB4* or *virD4* genes in *B. tribocorum* prevents the bacterium from establishing intraerythrocytic bacteraemia in a rat model of infection[45] — although whether this failure is due to changes in endothelial cell interaction or the inability to deliver a necessary effector protein remains to be determined. It is notable that another family of zoonotically acquired human pathogens, *Brucella*, which causes undulant fever in humans, also requires a type IV secretion system to establish infection[46].

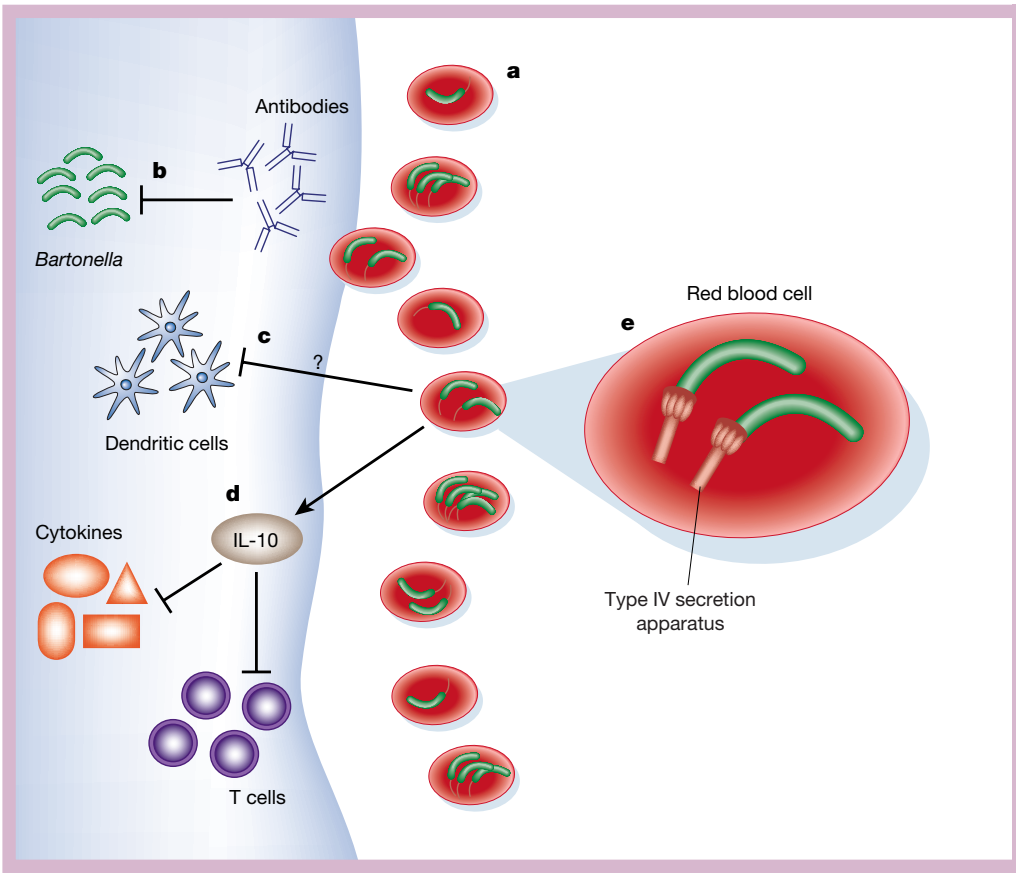


**Figure 2** Proposed strategies by which *Bartonella* establishes persistent infection. **a**, *Bartonella* colonizes an unknown niche within the body and seeds bacteria that are able to infect and survive inside red blood cells. **b**, Host antibodies have no effect on intraerythrocytic bacteria but prevent new waves of erythrocytic parasitism. **c**, **d**, Intracellular bacteria have been proposed to affect dendritic cell maturation through an unknown mechanism (**c**), and have been shown to inhibit cytokine production and T-cell proliferation (**d**) by inducing the expression of interleukin-10 (IL-10). **e**, A type IV secretion system is essential for establishing persistent intraerythrocytic infection.

Box 3
**What's in a name?**

It's unlikely that you've ever heard the name Mary Mallon. Mary was a young Irish woman who emigrated to the United States in 1883 in search of a better life. You may be familiar with 'Typhoid Mary', the moniker by which Mary Mallon came to be known. The legend of Typhoid Mary has grown over the years and she is reputed to have caused hundreds, if not thousands, of deaths in turn-of-the-century New York. In reality, she was probably responsible for only 33 cases of typhoid fever, resulting in three deaths.

Typhoid fever is caused by the bacterium *Salmonella typhi* and is characterized by a high fever, powerful headaches, nausea, diarrhoea and tenderness of the abdomen. The bacterium can be spread easily between humans via a faecal–oral route, and in the 1800s typhoid killed one in every ten people who were infected and so was feared perhaps as much as the plague.

However, *S. typhi* also has the ability to colonize some people without causing any disease symptoms. Unknown to Mary, who worked as a cook for a number of different families, she was a carrier of this deadly bacterium. Because *S. typhi* can be spread easily, Mary's constant handling of uncooked foods with improperly cleaned hands led her to cause typhoid outbreaks in seven of the eight families that she worked for, as well as in a hospital where she worked for a short while. Her carrier status was discovered in 1906 by George Soper, a sanitary engineer who was hired to determine the source of the mysterious typhoid outbreaks that seemed to appear wherever Mary was working.

As one of the first recorded typhoid carriers in the United States, Mary became reviled for her inability to understand how she could be spreading disease when she wasn't unwell herself, and for her refusal to stop cooking, which was her only means of financial support at the time. Eventually, she was exiled to the Riverside Hospital on North Brother Island, New York, where she lived in virtual isolation for 26 years and died of complications from a stroke in 1938. Her memory has lived on, however, and now the name Typhoid Mary is synonymous with death and disease, despite the fact that Mary Mallon started out as a simple, apparently healthy woman who was just trying to make a living.

In addition to its ability to invade and persist within red blood cells, *Bartonella* apparently manipulates the host immune system through modification of host-cell cytokine production. It was shown that homeless people who were chronically and asymptomatically infected with *B. quintana* showed decreased levels of circulating markers of leukocyte activation and decreased cytokine production by mononuclear cells. This was accompanied by an increase in the secretion of interleukin-10 (IL-10)[43], which depresses the expression of other cytokines and the release of soluble inflammatory mediators. Activation of T cells in the presence of IL-10 has been shown to result in non-responsiveness of these cells and attenuation of downstream signalling events[47]. Thus *Bartonella*-induced expression of IL-10 may directly facilitate bacterial persistence. This increasingly recognized persistence strategy has been observed for *Leishmania major*, *Coxiella burnetii*, *Yersinia pseudotuberculosis* and *Onchocerca volvulus*, the causative agents of leishmaniasis, Q fever, gastroenteritis and river blindness, respectively. Like *Bartonella*, these pathogens not only circumvent normal host defences, but also directly manipulate them.

## Fighting back

Years of research have helped to define our current understanding of pathogens and host–pathogen interactions. But of the more than 1,400 known bacterial, viral and parasitic pathogens that infect humans, we have managed to completely eradicate only smallpox[48]. Although the spread of several pathogens has been greatly diminished over the past 30 years, a further 37 human pathogens have been discovered and an estimated 12% of the known pathogens have been recognized as emerging or re-emerging[49]. About 200 different bacterial species are known to cause human disease[48], and these microbes use multiple and diverse strategies to overcome the host immune system. With the continuing rise in infectious-disease-related morbidity and mortality, future research should be directed towards gaining a greater understanding of the host–pathogen interface and strategies used by microbes for modulation of the host immune system. To accomplish this, we must focus on clarifying not only virulence factors *per se*, but also specific mechanisms that allow microbes to persist within the host environment. Gaining a thorough knowledge of chronically infecting microbes and learning how to control them is perhaps the greatest challenge. It is our belief that this should be made a major research focus and funding priority. We must strive to develop and apply novel technological approaches and new model systems to the elucidation of persistence strategies if we ever hope to find ways to subvert these stealthy pathogens. □

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