Yersinia pestis and plague

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Abstract

Yersinia pestis is the aetiological agent of plague, a disease of humans that has potentially devastating consequences. Evidence indicates that Y. pestis evolved from Yersinia pseudotuberculosis, an enteric pathogen that normally causes a relatively mild disease. Although Y. pestis is considered to be an obligate pathogen, the lifestyle of this organism is surprisingly complex. The bacteria are normally transmitted to humans from a flea vector, and Y. pestis has a number of mechanisms which allow survival in the flea. Initially, the bacteria have an intracellular lifestyle in the mammalian host, surviving in macrophages. Later, the bacteria adopt an extracellular lifestyle. These different interactions with different host cell types are regulated by a number of systems, which are not well characterized. The availability of the genome sequence for this pathogen should now allow a systematic dissection of these regulatory systems.

Introduction

Yersinia pestis is the aetiological agent of plague, a disease which has occurred in three pandemics and which, at least in Europe, has shaped social structures and even the genetic makeup of the population [1]. The most notorious of these outbreaks is the so-called ‘Black Death’ pandemic, which occurred in Europe between the 14th and 16th centuries. Even today, at least 2000 cases of plague are reported annually to the World Health Organization (WHO), and the outbreak of pneumonic plague in Surat, in India, reminded the world of the potential for this disease to spread rapidly. Most of the cases of disease in humans that occur nowadays are bubonic plague, which is usually the consequence of the bite from a flea that has previously fed on a rodent infected with Y. pestis. It is this form of the disease that gives rise to the classical symptom of plague: the swelling of the local draining lymph node (or bubo), usually in the groin or armpit. Occasionally, the infection spreads beyond this focus of infection to the bloodstream. Such cases of septicaemic plague are difficult to treat and often result in the colonization of the lungs. A subsequent secondary pneumonia can result in disseminated bacteria by the airborne route, as a consequence of coughing. It is these airborne bacteria that pose the greatest risk to human health, because their inhalation can result in primary pneumonic plague. Pneumonic plague is notoriously difficult to treat because of the speed with which the disease develops (typically the incubation period is 1–3 days), and also because, by the time individuals are symptomatic, they are often close to death.

The genome sequence of Y. pestis has recently been determined. This information has provided new insight into the evolution of this pathogen. In addition, genome sequence and other information provides insight into the ways in which this pathogen rapidly changes its lifestyle to adapt to survival in fleas and to growth, first in macrophages and later outside of host cells. The present paper provides an overview of the evolution of this pathogen and the consequences of these evolutionary changes for the lifestyle of the pathogen.

Y. pestis evolved from a low-grade enteric pathogen

All of the available evidence indicates that Y. pestis evolved between 1500 and 20 000 years ago from Yersinia pseudotuberculosis [2], a pathogen which causes a relatively mild enteric disease in humans. A range of biotypes of Y. pseudotuberculosis are known to exist, differing mainly in their make-up of the lipopolysaccharide O-antigen. There are several mutations in the O-antigen cluster in Y. pestis and, as a consequence, an O-antigen is not produced [3]. However, this cluster shows an overall make-up that is similar to that found in Y. pseudotuberculosis biotype 1b [3]. On this basis, Y. pseudotuberculosis 1b has been suggested to be the ancestral biotype of Y. pestis.

The evolution of Y. pestis appears to have occurred as the consequence of the loss of several functions associated with enteric disease, and the acquisition of a range of new functions that are encoded primarily on two additional plasmids (pFra and pPcp) [4]. Y. pestis also exploits some of the virulence mechanisms found in Y. pseudotuberculosis, but to greater effect. The origins of pFra and pPcp are not clear; however, a plasmid with a high degree of overall sequence identity with pFra has been found in some isolates of Salmonella typhi (the aetiological agent of typhoid fever) from Vietnam [5]. At this time, it is not clear whether these plasmids have been acquired from a common ancestor (or donor) or whether these plasmids have been transferred between Y. pseudotuberculosis and S. typhi.
Pathogenesis of plague

Genes that allow the colonization of the flea

The classical insect vector for *Y. pestis* is the flea, usually *Xenopsylla cheopis*. The flea becomes infected with bacteria as a consequence of feeding on a rodent infected with *Y. pestis*. Within the flea, the blood meal coagulates, blocking the foregut of the flea and thus preventing digestion of the blood meal. A number of proteins appear to contribute to the survival of *Y. pestis* in the flea. The chromosomally borne haemin storage (*hms*) locus encodes a number of membrane proteins that result in the pigmentaton of *Y. pestis* when cultured *in vitro* on agar containing haemin or Congo Red dye. The ability of bacteria to bind haemin led to initial speculation that the Hms proteins might form part of an iron-acquisition system. However, *hms* mutants of *Y. pestis* are fully virulent in the murine model of disease and the Hms system does not appear to function as a system for the acquisition of nutritionally accessible iron [6]. In the flea, the Hms proteins appear to alter the hydrophobicity of the bacterial cell, thereby promoting aggregation and clumping of bacteria within the blood meal [7]. This may be one of the main mechanisms by which blocking of fleas occurs. The *Y. pestis* phospholipase D plays a key role in promoting survival of bacteria within the flea gut, and a phospholipase D mutant of *Y. pestis* was unable to cause flea blocking [8]. The precise role of this enzyme is not known, but it may have a role in the restructuring of the bacterial cell wall, thereby protecting bacteria from serum-derived cytotoxins [8].

Genes that allow growth and regulate survival in the macrophage

The bite of a mammal from a flea that carries *Y. pestis* results in the regurgitation of the bolus of *Y. pestis* cells, which are embedded in the coagulated blood meal. As a consequence of the flea bite, it has been suggested that up to 24 000 *Y. pestis* cells are delivered intradermally into the new mammalian host [1]; these bacteria are readily ingested by professional phagocytes. Bacteria that are ingested by neutrophils appear to be readily killed, but bacteria within macrophages are able to survive and proliferate. The determinants which allow survival and growth in the macrophage are not known; however, *Y. pestis* has been shown to possess a two-component regulatory system, which is closely related to the PhoP/PhoQ two-component regulatory system in *Salmonella typhimurium* (a pathogen of humans that causes a relatively mild gastrointestinal disease). Like *S. typhimurium*, the *Y. pestis* PhoP/PhoQ system appears to regulate survival in macrophages, and also like *S. typhimurium*, extracellular Mg$^{2+}$ levels might activate this system. A *phoP* mutant of *Y. pestis* is moderately attenuated in the murine model of disease, but, more importantly, this mutant shows an enhanced susceptibility to macrophage killing mechanisms, and has reduced survival in macrophages [9]. A number of genes appear to be regulated by the PhoP/PhoQ system [9], but their identities have not yet been determined, although some of the gene products are thought to have a role in the modification of lipo-oligosaccharide [10]. The ability of *Y. pestis* to survive in macrophages is critical to the early pathogenesis of disease, and bacteria within macrophages appear to be trafficked to the local draining lymph node. Within these lymph nodes, a massive infiltration of phagocytic cells occurs, resulting in the formation of a bubo.

Genes that allow growth and replication outside of host cells

During growth in macrophages and trafficking to the lymph node, another key regulatory system modulates the properties of the bacteria. Bacteria within the flea are incubated at temperatures below 37°C. The exposure to temperatures of around 37°C in the mammalian host results in the up-regulation of a range of virulence factors. Within the bubo, and by an unknown mechanism, the bacteria appear to escape from infected macrophages and to adopt an extracellular lifestyle. Some of the virulence determinants responsible for this temperature-dependent lifestyle switch have been identified. A polypeptide destined to form a surface capsule (F1-antigen) is produced in large amounts. This polypeptide is exported on to the cell surface where it appears to auto-assemble into fibrillar-like structures [11]. Mutants of *Y. pestis* that are unable to produce F1-antigen show an enhanced susceptibility to phagocytosis by macrophages [12].

Possibly, the key virulence mechanism that allows the bacteria to resist further phagocytosis is the type III secretion system (Figure 1). The type III secretion system is up-regulated at 37°C, i.e. within the mammalian host. This system allows bacteria that are in contact with host cells to inject a range of so-called effector proteins (or effector *Yersinia* outer proteins, or Yops) into the host cell via the secretory apparatus [13,14]. The functions of the effector Yops fall broadly into two groups: YopH and YopT target the cytoskeleton by dephosphorylating paxillin/focal adhesion kinase and by modifying RhoA GTPase respectively [13–15]. YopE also disrupts the cytoskeleton by an unknown mechanism. In contrast, YopJ (alternatively known as YopP) down-regulates the inflammatory response and induces apoptosis in macrophages [13,14]. Although the type III system in *Y. pestis* is thought to be activated on contact with host cells, and at 37°C, the molecular events which transduce the cell-contact signal are not known. However, some of the events which occur in the bacterium on activation of the type III system are understood. In the repressed state, the type III secretory apparatus system is thought to be ‘blocked’ on the outside by the YopN protein, and on the inside of the bacterial cell by the protein LcrG. Before contact with the host cell, the expression of Yops is repressed by the accumulation of LcrQ within the bacterium. Induction of the type III system by contact with a eukaryotic host cell triggers a cascade of events. In one model, YopN is then released from the cell surface [16]. Although the secretory system is still partially blocked by LcrG, there is sufficient release of LcrQ to allow the induction of Yop.
expression. One of the first Yops to be produced is LcrV. LcrV is thought to bind with high affinity to LcrG [17], which otherwise blocks the secretory apparatus. Titration of LcrG by LcrV completely unblocks secretion. The VirF protein, which is now produced, serves as a positive activator of Yop expression. Next, the various Yops that are required for pore formation in the host cell (YopB and YopD) are produced, and the effector Yops are translocated into the eukaryotic host cell. Our recent results suggest that the role of LcrV in the regulation of the type III system is more complex than was originally thought, and that LcrV might interact with other components of the type III system in a regulatory capacity [17].

Clearly, LcrV within the bacterial cell is important for the activation of the type III system. LcrV is also found on the cell surface [18] and is also exported from the bacterial cell. The function of surface-located LcrV is not known. However, soluble LcrV appears to act on host cells to repress the production of the pro-inflammatory cytokines such as tumour necrosis factor α and interferon γ [13].

Conclusions
Although *Y. pestis* is considered to be an obligate pathogen, it shows a remarkable ability to survive and grow in a diversity of host environments. These interactions with different host cells are regulated by a number of mechanisms, which are only now being characterized at a molecular level. The availability of the genome sequence of this pathogen will now allow these complex regulatory networks to be characterized, for example using microarray technology. In the future, we might also expect further insights into genes which regulate or play a direct role in the pathogenesis of pneumonic plague.

References

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