New study sheds light on how Salmonella spreads in the body

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Findings of Cambridge scientists, published today in the journal *PLoS Pathogens*, show a new mechanism used by bacteria to spread in the body with the potential to identify targets to prevent the dissemination of the infection process.

Salmonella enterica is a major threat to public health, causing systemic diseases (typhoid and paratyphoid fever), gastroenteritis and non-typhoidal septicaemia (NTS) in humans and in many animal species worldwide. In the natural infection, salmonellae are typically acquired from the environment by oral ingestion of contaminated water or food or by contact with a carrier. Current vaccines and treatments for S. enterica infections are not sufficiently effective, and there is a need to develop new therapeutic strategies.

Dr Andrew Grant, lead author of the study from the University of Cambridge, said: "A key unanswered question in infectious diseases is how pathogens such as Salmonella grow at the single-cell level and spread in the body. This gap in our knowledge is hampering our ability to target therapy and vaccines with accuracy."

During infection, salmonellae are found mainly within cells of the immune system where they are thought to grow and persist. To do so the bacteria adapt to their surrounding environment and resist the antimicrobial activity of the cell. Research from the Cambridge group has shown that the situation is more complex in that the bacteria must also escape from infected cells to spread to distant sites in the body,

avoiding the local escalation of the immune response and thus playing a 'catch me if you can' game with the host immune system.

A body of knowledge has been built using in vitro (test tube) cell culture experiments that indicates that replication of *Salmonella enterica* within host cells in vitro is somewhat dependent on the bacteria making a syringe-like structure, called a Type 3 Secretory System (T3SS). This then injects bacterial proteins into the host cell, which in turn enhance bacterial replication inside that cell. This T3SS is encoded by genes in a region of the bacterial chromosome called Salmonella Pathogenicity Island 2 (or SPI-2). Translating this cell culture work into whole animals, it has become accepted dogma that the SPI-2 T3SS is also required for bacterial intracellular replication in cells inside the body.

However, using fluorescence and confocal microscopy (which are imaging techniques), the Cambridge team has dispelled this dogma concerning the requirement for the SPI-2 T3SS for intracellular replication in the body. The researchers have shown that mutants lacking SPI-2 can reach high numbers within individual host cells, a situation that does not happen in in vitro cell culture.

The researchers, from the Mastroeni and Maskell laboratories at the University of Cambridge's Department of Veterinary Medicine, investigated this phenomenon further and made the surprising discovery that salmonellae lacking the SPI-2 T3SS remain trapped inside cells and cannot spread in the body. One idea is that this will in turn lead to the arrest of bacterial division as a consequence of spatial or nutritional constraints. Despite growing to high numbers per cell, these mutants are much less able to grow overall in the body because far fewer cells become infected due to the greatly reduced ability of the bacteria to escape from the original infected cells.

These findings call into question the usefulness of some in vitro

experimental systems that, when used in isolation, do not usefully represent the very complex structure of mammalian organisms.

The team also presented a new role for the NADPH phagocyte oxidase (Phox) (a host mechanism which generates reactive oxygen species which can inhibit the growth and/or kill the bacteria) in the control of *Salmonella* infection. They observed that this system inhibits bacterial escape from host cells, and that normally the SPI-2-encoded T3SS counters this system to facilitate bacterial exit from infected cells. This highlights a previously unknown interplay between SPI-2 T3SS and innate immunity in the dynamics of within-host bacterial growth and spread. The research shows that in the absence of an active Phox, SPI-2 T3SS becomes dispensable for the spread of *Salmonella* in the tissues. Conversely, when an active Phox is present, a SPI-2 T3SS mutant grows inside cells to high intracellular densities but appears to be unable to escape from the cells and disseminate in the body.

Dr Grant said: "*Salmonella* is a significant public health threat. Unfortunately, effective treatments and vaccinations have thus far eluded scientists, in part because of a lack of understanding of how and why the bacteria spread. This research provides critical insight which will hopefully lead to new medical interventions for this disease."

Provided by University of Cambridge