Modelling the interactions of Salmonellae with the human host using stem cell-derived intestinal organoids and macrophages

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Abstract

Salmonellae are Gram-negative, predominantly flagellated, facultative intracellular bacteria, and are an important cause of enteric disease in humans and in animal hosts worldwide. Their transmission is predominantly via the faeco-oral route and members of the *Salmonella enterica* species can be arbitrarily classified into typhoidal and non-typhoidal types based on their pathogenicity in a particular host. *Salmonella enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* serovar Paratyphi A (*S.* Paratyphi A) cause typhoid and paratyphoid fevers respectively, which are collectively associated with ~25 million cases and 250,000 deaths per year, predominantly concentrated in regions of Asia and Africa where sanitation and clean water are difficult to access. Non-typhoidal serovars (NTS) (e.g. *S. enterica* serovar Typhimurium (*S.* Typhimurium), *S. enterica* serovar Enteritidis (*S*. Enteritidis)) are responsible for over 93 million infections and ~155,000 deaths worldwide per year, the majority of which are thought to be food-borne infections. NTS infections usually cause self-limiting gastroenteritis, but can progress to invasive disease (iNTS) with bacteraemia and a mortality rate as high as 25% in certain patients. *S.* Typhi and *S*. Paratyphi A are pathogens restricted to humans, meaning that there has been difficulty until recently in producing a valid model with which to study pathogen-mucosal interactions and learn more about the invasion mechanisms of these unique pathogens. Although *S.* Typhimurium is predominantly associated with a localised gastroenteritis in immunocompetent humans; it causes a typhoid-like disease in mice; therefore mouse models have previously been used as surrogates to provide concepts about the interaction between *S.* Typhi and the host mucosa and resultant immune response.

However, in recent years, a new approach to studying pathogen-gut epithelial interactions has been developed; known as “organoids” or human intestinal organoid systems (iHO).

These can be produced from human induced pluripotent stem cells (hiPSCs), or from primary intestinal tissue, and once matured, harbour differentiated enterocytes and secretory cells such as goblet, Paneth and enteroendocrine cells. They have previously proved capable of providing a complementary human model for studying *S.* Typhimurium infection, but their utility has been explored during this project to include the human gut epithelial interaction with other serovars of *Salmonella* (in particular typhoidal strains, which have never been studied in this context) and the transcriptomic/phenotypic response to these enteric pathogens.

In vivo, intestinal epithelial cells (IECs) play a key role in regulating intestinal homeostasis, and can directly inhibit pathogens, although the mechanisms by which this occurs are not well understood. I have demonstrated that the cytokine IL-22 has a role in IEC defence against *S.* Typhimurium in the hiPSC-derived iHO system, with evidence for restriction of intracellular infection of wild type *S*. Typhimurium SL1344 in iHO pre-treated with recombinant human IL-22. I have demonstrated that a mechanism via which this protection occurs is increased phagolysosomal fusion. I have also modelled infections with alternative types of bacteria, including *S*. Enteritidis and enteropathogenic *Escherichia coli* (EPEC); investigating whether luminal killing of bacteria occurs within the iHO system. I have used iHO derived from stem cells with induced mutations to explore genes of interest in epithelial defence. Lastly, I have demonstrated that typhoid-causing Salmonellae (*S*. Typhi and *S*. Paratyphi A) are able to invade both the iHO epithelium and hiPSC-derived macrophages from the same cell line. I investigated these interactions using imaging and bulk RNA-Seq to identify differences in response to the bacteria in the epithelial and immune cell compartments. Strikingly, genes differentially expressed in IECs showed most similarities in response to infection with non-encapsulated serovars (*S*. Paratyphi A and *S*. Typhimurium versus *S*. Typhi), whereas genes differentially expressed in macrophages demonstrated most overlap in response to typhoid-causing strains (*S*. Typhi and *S*. Paratyphi A versus *S*. Typhimurium), raising important questions about the immunomodulatory role of the Vi capsule and the apparent ability of *S*. Paratyphi to behave differently in the epithelial and macrophage environments. It was also possible to demonstrate that H58 serovars of *S*. Typhi caused distinct transcriptional signatures in the macrophage model, versus their non-H58 counterparts. I present this novel data and discuss how this complements what is currently known about the host-pathogen interactions of typhoid-causing Salmonellae.