

Host factors involved in intracellular growth and proliferation of *Salmonella*

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Introduction: *Salmonella* spp. are facultative intracellular pathogens of animals and humans which survive and proliferate within an intracellular phagosome/vacuole in infected host cells. While a great deal is known about the genes and processes involved in invasion and establishment of the intracellular phagosomal niche, almost no information is available concerning the intracellular proliferative phase of infection. From within the phagosome, *Salmonella* is known to modulate host cell endosomal trafficking, and shows at least transient interactions with a number of different endosomal compartments, suggesting *Salmonella* has access to endosomes or vesicles containing metabolizable compounds for intracellular growth (1,2,3). However, the acquisition of nutrients is not simply a matter of fusion with endocytic vesicles since intracellular, phagosomal *Salmonella* are protected from the action of extracellular antibiotics which would also be present in these endocytic vesicles. Studies in our laboratory indicate that up to 70% of the intracellular growth yield of *Salmonella* is dependent on access to host-dependent pools of amino acids or serum proteins. A recent study with another intracellular pathogen, *Legionella pneumophila*, identified the host neutral amino acid transporter, SLC1A5, as necessary for intracellular growth of *Legionella* (4), suggesting that acquisition of amino acids or peptides from the host may be a common feature of phagosome-bound, intracellular pathogens. Furthermore, amino acids which feed into central carbon pathways of the bacterium appear to limit the intracellular growth of *Salmonella* (5).

Previous work: Studies in our laboratory showed that *de novo* amino acid biosynthesis is not required for intracellular growth of *Salmonella*, indicating that phagosomal *Salmonella* has access to host pools of amino acids. These results indicate that post-infection, intracellular *Salmonella* utilizes host cell pools of amino acids or peptides for growth, either by accessing endocytic pathways or other, internal host pools. As phagosomal *Salmonella* are protected from antibiotics present in endocytic vesicles, the intracellular host pools of amino acids and/or peptides which are accessed by *Salmonella* must be derived from host cell amino acid and peptide uptake systems which selectively transport these metabolites into the cell.

Work hypothesis: Intracellular, phagosomal *Salmonella* acquire amino acids and/or peptides from the host for intracellular growth, and *Salmonella* must compete with the host cell for these metabolites. Although *Salmonella* is prototrophic, *i.e.* capable of biosynthesis of amino acids from simple carbon compounds, amino acid auxotrophs of *Salmonella* remain completely dependent upon the host for amino acids and therefore dependent upon host uptake and transport systems. The observation that amino acid auxotrophs of *Salmonella* still successfully proliferate within the host indicates that the pathogen is capable of diverting amino acid and/or peptide pools for its own use. We will use the host-dependence of amino acid auxotrophs to identify the host cell uptake and transport systems used by *Salmonella* for intracellular growth.

Work Plan: Amino acid and uptake mutants of *Salmonella typhimurium* which are completely dependent upon the host for amino acids and/or peptides for intracellular growth have been constructed. The project will consist of using specific inhibitors of eukaryotic amino acid and peptide transporters (6) to identify the host transporters involved in supplying *Salmonella* with intracellular pools of amino acids. Both published (4) and new siRNA constructs will also be used to inhibit the expression of host amino acid transporters to determine the roles of individual host transport systems on bacterial growth. We have previously used targeting sequences fused to fluorescent proteins to determine the extent of co-localization with Golgi, ER, mitochondrial and other compartments, and other constructs are available for the *trans*-Golgi network. Endosomal compartment targeting of amino acid

analogue derivatives or toxic peptides such as tri-L-ornithine or glycyl-glycyl- derivatives fused to endosomal targeting sequences will be used to determine the accessibility for *Salmonella* to various host cell compartments for intracellular growth. This work could have implications for directed targeting of inhibitory compounds against membrane-bound, intracellular phagosomal pathogens.

Proposed thesis topics (Ph.D. students):

- (1) The role of host cell amino acid and peptide transporters on intracellular growth of *Salmonella typhimurium*.
- (2) Intracellular sources of host metabolite pools for growth of phagosomal *Salmonella*

References:

- (1) Salcedo, S.P. and Holden, D.W. (2005) Bacterial interactions with the eukaryotic secretory pathway. *Curr. Opin. Microbiol.* 8:92-98
- (2) Abrahams, G.L. and Hensel, M. (2006) Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cell. Microbiol.* 8:728-737
- (3) Ramsden, A.E., Holden, D.W. and Mota, L.J. (2007) Membrane dynamics and spatial distribution of *Salmonella*-containing vacuoles. *Trends Microbiol.* 15:516-524
- (4) Wieland, H., Ullrich, S., Lang, F., and Neumeister, B. (2005) Intracellular multiplication of *Legionella pneumophila* depends on host cell amino acid transporter SLC1A5. *Mol. Microbiol.* 55:1528-1537
- (5) Tedin, K. and F. Norel. (2001) Comparison of \square *relA* strains of *Escherichia coli* and *Salmonella typhimurium* suggests a role for ppGpp in attenuation regulation of branched chain amino acid biosynthesis. *J. Bacteriol.* 183:6184-6196.
- (6) Bröer, Stefan (2008) Amino acid transport across mammalian intestinal and renal epithelia. *Physiol. Revs.* 88:249-286.