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Low-oxygen tensions found in *Salmonella*-infected gut tissue boost *Salmonella* replication in macrophages by impairing antimicrobial activity and augmenting *Salmonella* virulence

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Abstract:

In *Salmonella* infection, the *Salmonella* pathogenicity island-2 (SPI-2)-encoded type three secretion system (T3SS2) is of key importance for systemic disease and survival in host cells. For instance, in the streptomycin-pretreated mouse model SPI-2-dependent *Salmonella* replication in lamina propria CD-11c^{-}CXCR1^{-} monocytic phagocytes/macrophages (MΦ) is required for the development of colitis. In addition, containment of intracellular *Salmonella* in the gut critically depends on the antimicrobial effects of the phagocyte NADPH oxidase (PHOX), and possibly type 2 nitric oxide synthase (NOS2). For both antimicrobial enzyme complexes, oxygen is an essential substrate. However, the amount of available oxygen upon enteroinvasive *Salmonella* infection in the gut tissue and its impact on *Salmonella*-MΦ interactions was unknown. Therefore, we measured the gut tissue oxygen levels in a model of *Salmonella* enterocolitis using luminescence two-dimensional *in vivo* oxygen imaging. We found that gut tissue oxygen levels dropped from ~ 78 Torr (~ 11 % O_2) to values of ~ 16 Torr (~ 2 % O_2) during infection. Because *in vivo* virulence of *Salmonella* depends on the *Salmonella* survival in MΦ, *Salmonella*-MΦ interaction was analysed under such low oxygen values. These experiments revealed an increased intracellular replication and survival of wild-type t3ss2 non-expressing *Salmonella*. These findings were paralleled by blunted nitric oxide and reactive oxygen species (ROS) production and reduced *Salmonella* ROS perception. In addition, hypoxia enhanced SPI-2 transcription and translocation of SPI-2-encoded virulence protein. Neither pharmacological blockade of PHOX and NOS2 nor impairment of T3SS2 virulence function alone mimicked the effect of hypoxia on *Salmonella* replication under normoxic conditions. However, if t3ss2 non-expressing *Salmonella* were used, hypoxia did not further enhance *Salmonella* recovery in a PHOX and NOS2-deficient situation. Hence, these data suggest that hypoxia-induce impairment of antimicrobial activity and *Salmonella* virulence cooperate to allow for enhanced *Salmonella* replication in MΦ.

Keywords: *Salmonella* infection, gut tissue oxygen level, in vivo imaging, intracellular replication, hypoxia, monocytic phagocytes/macrophages