Immune evasion and survival strategies of evolved hyper-virulent *Salmonella* Typhimurium strains

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Introduction: *Salmonella* is a Gram-negative, rod-shaped bacteria which causes millions of death worldwide due to gastroenteritis and typhoid fever in humans and animals. The mortality of *Salmonella* infection has gradually increased because of the emergence of antibiotic-resistant strains such as multidrug-resistant (MDR) and extremely drug-resistant (XDR). The multiple host-pathogen and non-host environment help in the emergence of multidrug-resistance and hypervirulent strains. Our previous study showed *Salmonella* Typhimurium (STM) adaptation to different *in-vitro* and *in-vivo*, and evolved into hypervirulent strains P12-STM, Ce12-STM, and F12-STM. This study deals with the mechanism of immune evasion strategies of these three strains and how they become hypervirulent and hyperproliferative inside mouse macrophage RAW-264.7.

Methodology: Real-time PCR, western blotting, and confocal microscope were used to check the immune evasion and alteration of signaling pathways by the hyper-virulent strains.

Results: The hypervirulent strains create an anti-inflammatory environment inside Raw-264.7 by altering cytokine production via NF-κB and Akt-NLRC4 signaling for their better survival inside mouse macrophage. The strain also reduced the lysosome-phagosome fusion for better survival in comparison to unpassaged wild-type strain.

Conclusion: The evolved hypervirulent *Salmonella* strains reduce the host inflammatory response by resulting in an anti-inflammatory environment by upregulating IL-10 and down-regulating IL-1β expression. Upon inhibition of NF-κB and Akt signaling, the cytokine expression and bacterial burden inside mouse macrophages reverted, which suggests that these hypervirulent strains modulate the host immune system through these pathways.

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INTRODUCTION

Salmonella is a Gram-negative, rod-shaped bacteria which causes millions of death worldwide due to gastroenteritis and typhoid fever in humans and animals. The mortality of *Salmonella* infection has gradually increased because of the emergence of antibiotic-resistant strains such as multidrug-resistant (MDR) and extremely drug-resistant (XDR). The *Salmonella* strains with increased infectivity have been reported from nature as well as from several host and non-host environments. The multiple host-pathogen and non-host environment help in the emergence of multidrug-resistance and hyper-virulent strains. There are few reports on *Salmonella* adaptation studied experimentally either by exposing it to different stresses like temperature, acid, salt, etc. through animal passaging studies or even from patient isolates which revealed that *Salmonella* is indeed undergoing adaptation ultimately altering its pathogenicity. Our previous study showed *Salmonella* Typhimurium (STM) adaptation to different *in-vitro* and *in-vivo*, and evolved into hyper-virulent strains P12-STM, Ce12-STM, and F12-STM. This study deals with the mechanism of immune evasion strategies of these three strains and how they become hyper-virulent and hyperproliferative inside mouse macrophage RAW-264.7.

METHODS

Mouse macrophage RAW-264.7 cells were infected with the WT-STM, and passaged strains (P12-STM, Ce12-STM, and F12-STM) with 10 MOI. Real-time PCR, western blotting, and confocal microscope were used to check the immune evasion and alteration of signalling pathways by the hyper-virulent strains. To further conform the signalling pathway the cells were treated with the NF-κB inhibitor with 50 nM and Akt inhibitor with 2 μM concentration for 60 and 30 min before the start of infection respectively followed by infection with the different *Salmonella* strains.

RESULTS

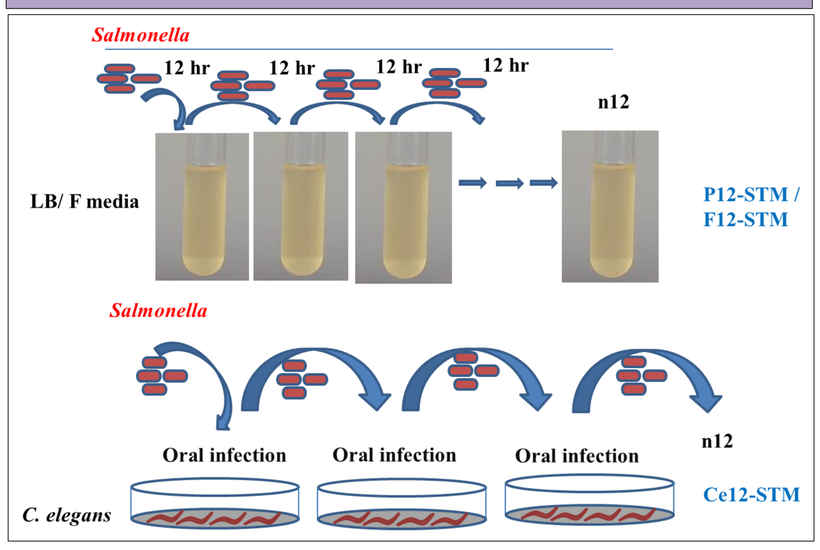

FIGURE 1. Repeated *in-vitro* and *in-vivo* passaging of *Salmonella* Typhimurium: Experimental set up

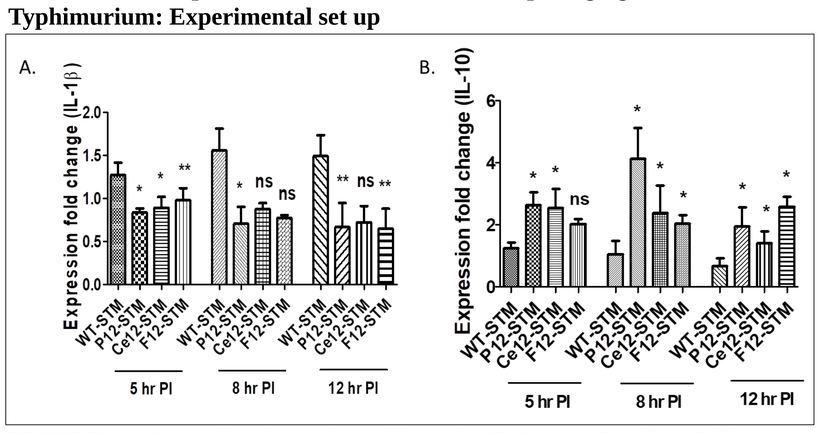

FIGURE 2. Quantification of cytokines in RAW-264.7 cells: Cells were infected with WT-STM, P12-STM, Ce12-STM and F12-STM with 10 MOI and expression level of IL-1 and IL-10 checked after 5, 8 and 12 hours PI.

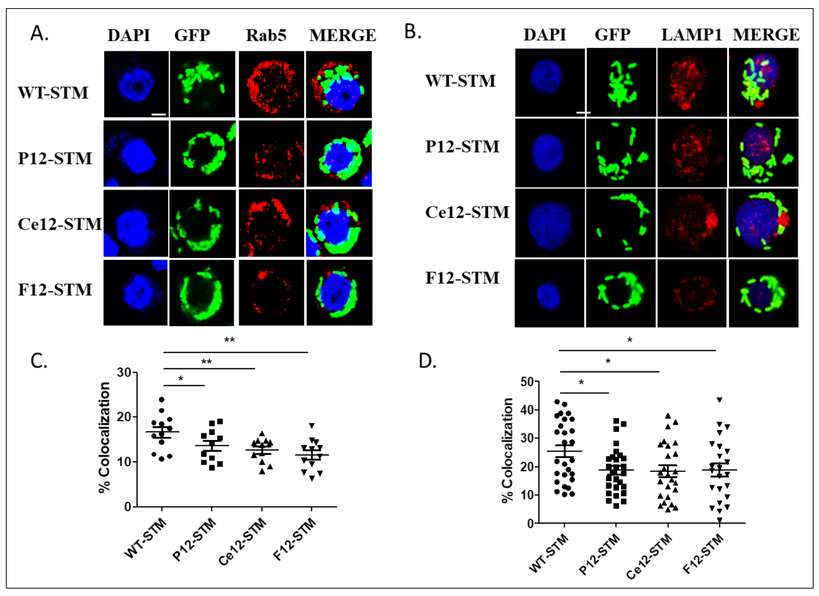

FIGURE 3. Colocalization of bacteria with lysosome in RAW-264.7 cells after 14 hours of PI

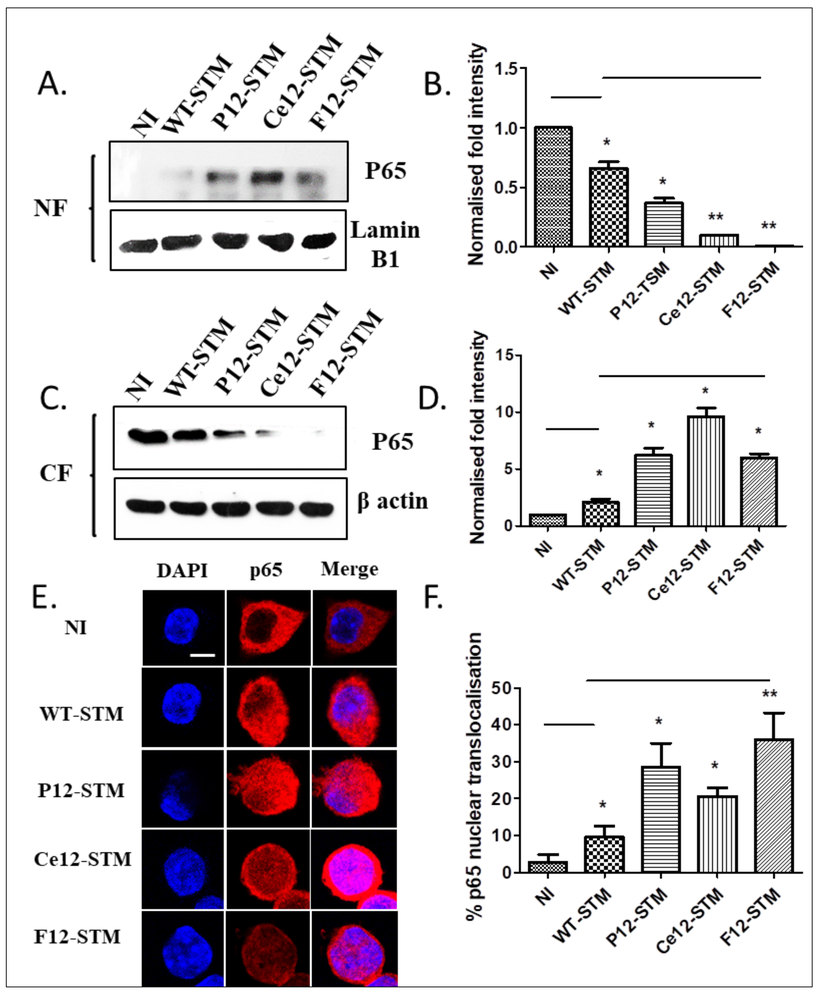

FIGURE 4. Modulation of the NF-κB pathway by the passaged strains in RAW-264.7 cells

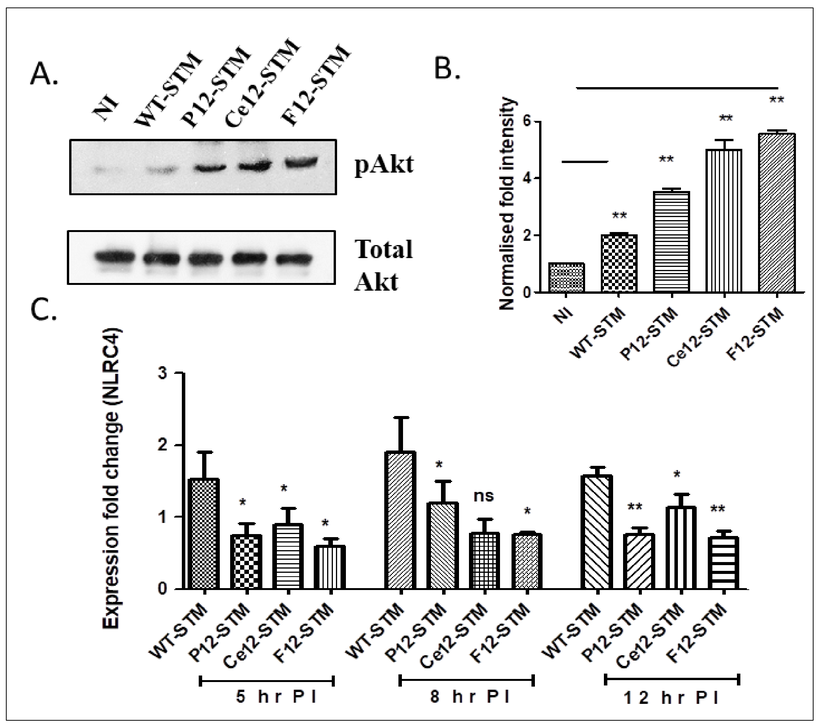

FIGURE 5. Akt phosphorylation in RAW-264.7 cells after 60 minutes of PI with passaged strains

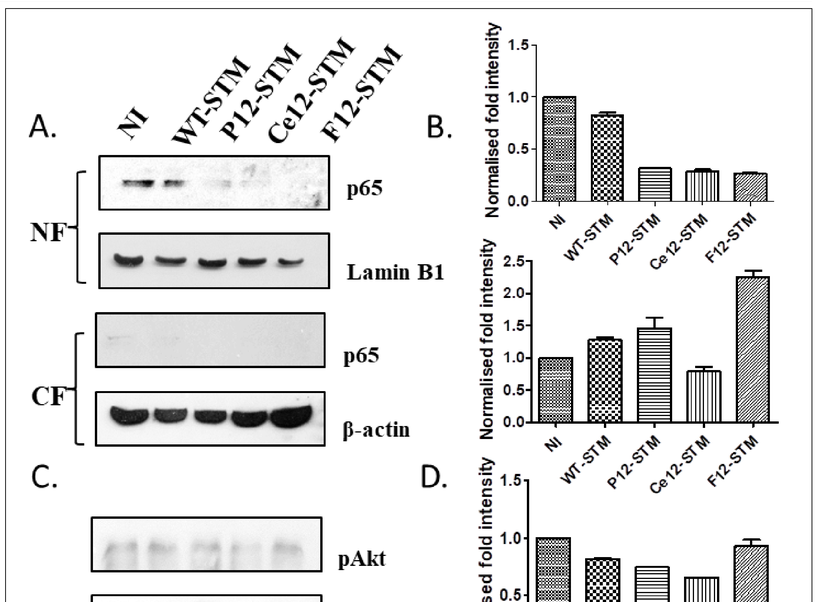

FIGURE 6. Inhibition of infection mediated NF-κB and Akt in Raw-264.7 cells using inhibitors

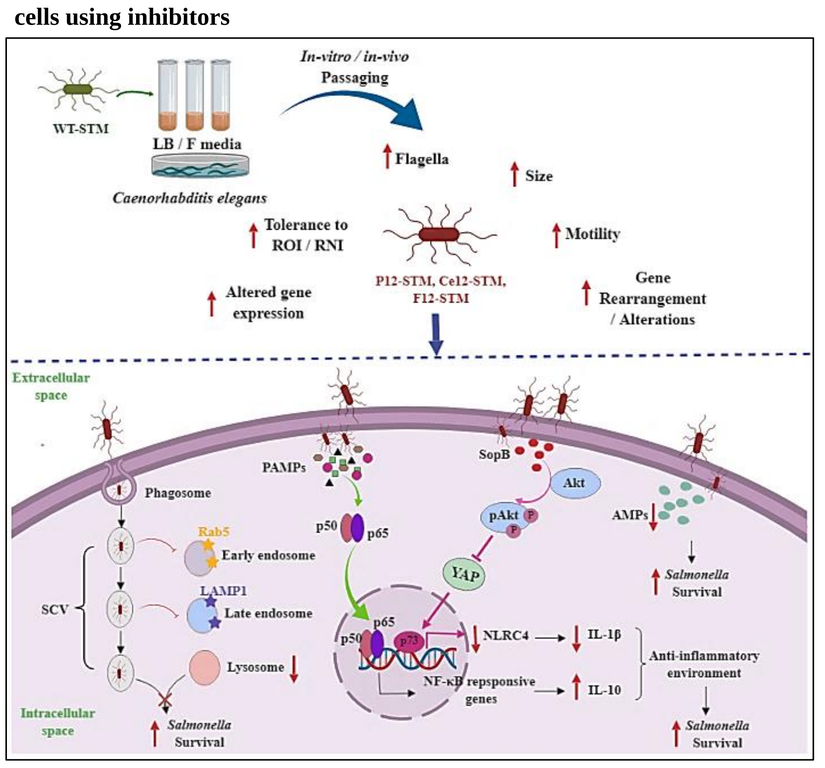

FIGURE 7. Evolutionary adaptations and immune evasion strategies by *Salmonella*: Pictorial representation of the emergence of hypervirulent *Salmonella* strains, P12-STM, Ce12-STM, and F12-STM through multiple passaging and their host immune modulation via inhibiting lysosomal degradation, altering pro and anti-inflammatory cytokines via NF-κB and Akt pathway and by reducing AMPs production

CONCLUSION

The evolved hyper-virulent *Salmonella* strains reduce the host inflammatory response by resulting in an anti-inflammatory environment by upregulating IL-10 and down-regulating IL-1β expression. Upon inhibition of NF-κB and Akt signalling, the cytokine expression and bacterial burden inside mouse macrophages reverted, which suggests that these hyper-virulent strains modulate the host immune system through these pathways.

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