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TLR Signaling Is Required for *Salmonella typhimurium* Virulence

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SUMMARY

Toll-like receptors (TLRs) contribute to host resistance to microbial pathogens and can drive the evolution of virulence mechanisms. We have examined the relationship between host resistance and pathogen virulence using mice with a functional allele of the *nramp-1* gene and lacking combinations of TLRs. Mice deficient in both TLR2 and TLR4 were highly susceptible to the intracellular bacterial pathogen *Salmonella typhimurium*, consistent with reduced innate immune function. However, mice lacking additional TLRs involved in *S. typhimurium* recognition were less susceptible to infection. In these TLR-deficient cells, bacteria failed to upregulate *Salmonella* pathogenicity island 2 (SPI-2) genes and did not form a replicative compartment. We demonstrate that TLR signaling enhances the rate of acidification of the *Salmonella*-containing phagosome, and inhibition of this acidification prevents SPI-2 induction. Our results indicate that *S. typhimurium* requires cues from the innate immune system to regulate virulence genes necessary for intracellular survival, growth, and systemic infection.

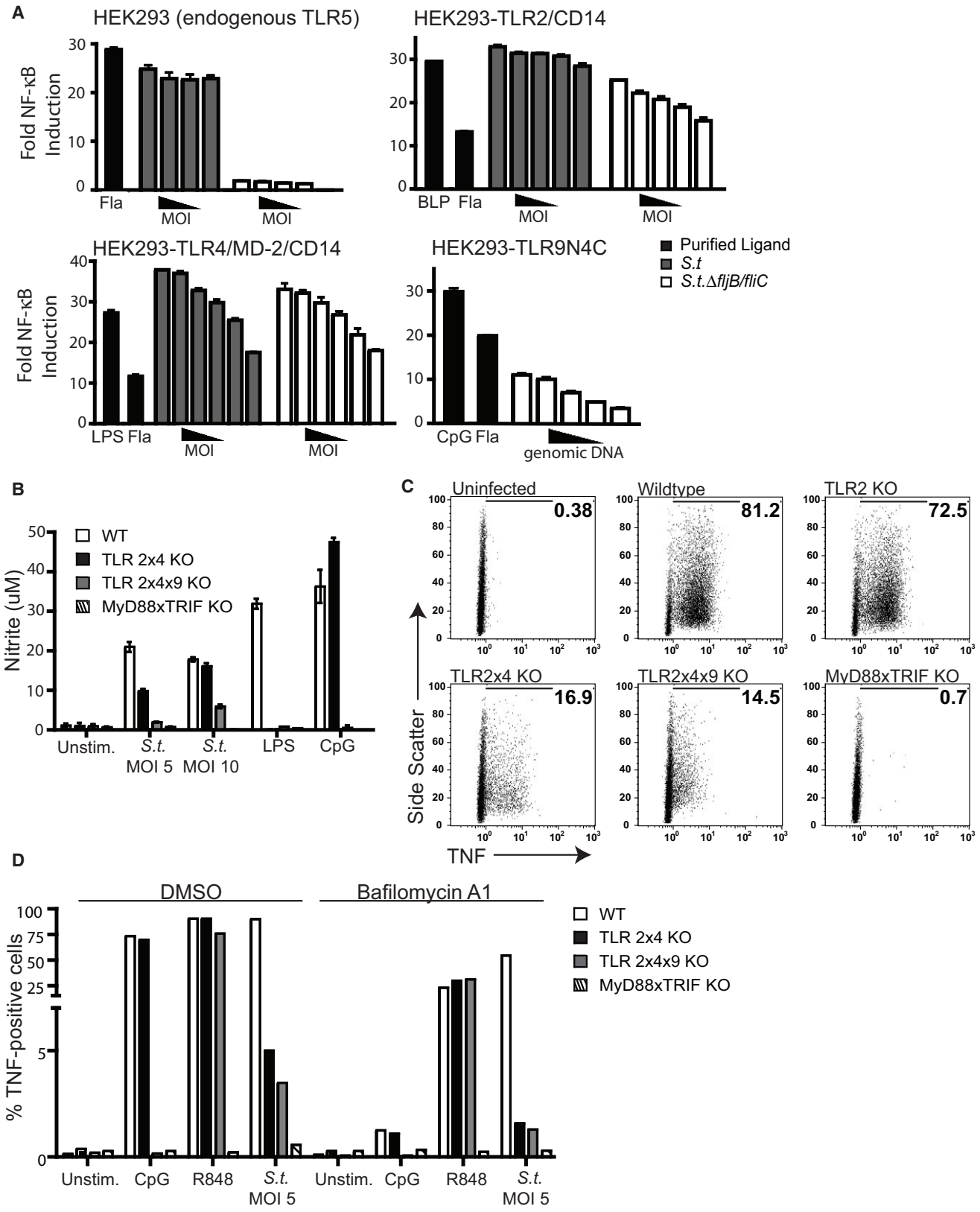
INTRODUCTION

During early stages of infection the innate immune system is essential for limiting microbial replication and spread before an adaptive response is mounted. Accordingly, pathogens have evolved virulence strategies to antagonize innate immune function (Hedrick, 2004; Rausher, 2001; Woolhouse et al., 2002). The interplay between host innate immune function and pathogen virulence mechanisms largely determines the outcome of most infections. Despite the logic of this conceptual framework, our understanding of the molecular interactions driving the emergence of virulence mechanisms remains relatively poor.

Innate immune receptors detect infection by recognizing conserved microbial features common to broad classes of microbes (Janeway, 1989; Medzhitov, 2007). The Toll-like receptors (TLRs) target a range of microbial ligands, including lipopolysaccharide (TLR4), lipoproteins (TLR2), flagellin (TLR5), unmethylated CpG motifs in DNA (TLR9), double-stranded RNA (TLR3), and single-stranded RNA (TLR7 and TLR8) (Akira et al., 2001; Kawai and Akira, 2005). Expression of TLRs on innate immune cells links microbial recognition to induction of antimicrobial mechanisms, such as production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and expression of antimicrobial peptides (AMPs). In addition, TLR activation can promote adaptive immunity through control of dendritic cell (DC) maturation (Iwasaki and Medzhitov, 2004).

To study the evolution of pathogen virulence and its relationship to innate immunity, we have focused on TLR-mediated recognition of *Salmonella enterica* serovar *typhimurium*. *S. typhimurium* is a gram-negative bacterium that can survive and replicate within host macrophages (Coburn et al., 2007). Survival within macrophages requires a set of genes, many of which are encoded within *Salmonella* pathogenicity island 2 (SPI-2) (Galan, 2001; Shea et al., 1996; Waterman and Holden, 2003). SPI-2 encodes a type 3 secretion system (T3SS) that is expressed after the bacterium is phagocytosed (Cirillo et al., 1998; Pfeifer et al., 1999; Valdivia and Falkow, 1997). Translocation of SPI-2 effectors into the host cell transforms the phagosome into a compartment that supports bacterial replication, the *Salmonella*-containing vacuole (SCV) (Marcus et al., 2000). Multiple signals have been implicated in the transcriptional induction of SPI-2, including cation deprivation, phosphate starvation, and low pH (Chakravortty et al., 2005; Cirillo et al., 1998; Deiwick et al., 1999; Kim and Falkow, 2004; Rappl et al., 2003). Most of the studies implicating these signals have been performed on bacteria grown in vitro; whether the same signals are responsible for induction of SPI-2 genes within the phagosome remains unclear.

Recognition of *S. typhimurium* is largely mediated by TLR2, TLR4, and TLR5 (Feuillet et al., 2006; Hapfelmeier et al., 2005; O'Brien et al., 1980; Royle et al., 2003; Smith et al., 2003; Uematsu et al., 2006; Vazquez-Torres et al., 2004). Consistent with a central role for these receptors, *S. typhimurium* has evolved mechanisms to subvert this recognition or to avoid the consequences of TLR activation. For example, modification of


A
HEK293 (endogenous TLR5)
HEK293-TLR2/CD14
HEK293-TLR4/MD-2/CD14
HEK293-TLR9N4C
Fold NF-κB Induction
Fla
BLP Fla
LPS Fla
CpG Fla
MOI
genomic DNA
Purified Ligand
S.t
S.t.ΔfljB/fliC
B
Nitrite (uM)
WT
TLR 2x4 KO
TLR 2x4x9 KO
MyD88xTRIF KO
Unstim.
S.t. MOI 5
S.t. MOI 10
LPS
CpG
C
Uninfected
Wildtype
TLR2 KO
TLR2x4 KO
TLR2x4x9 KO
MyD88xTRIF KO
0.38
81.2
72.5
16.9
14.5
0.7
Side Scatter
TNF
D
DMSO
Bafilomycin A1
% TNF-positive cells
WT
TLR 2x4 KO
TLR 2x4x9 KO
MyD88xTRIF KO
Unstim.
CpG
R848
S.t. MOI 5