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Proteolytic cleavage of NF-κB p65: a novel mechanism for subversion of innate immune signaling by pathogenic *E. coli*

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Enteropathogenic and enterohemorrhagic *Escherichia coli* (EPEC and EHEC, respectively) are attaching and effacing (A/E) bacterial pathogens that cause severe diarrheal disease. An integral mechanism in the virulence strategy of A/E pathogens is the ability to subvert innate immune signaling by down-regulation of interleukin (IL)-8, a pro-inflammatory chemokine that functions to recruit neutrophils to sites of infection (Vallance and Finlay, 2000). Expression of IL-8 is stimulated by several pro-inflammatory stimuli, which interact with cellular receptors and initiate signaling cascades terminating in the expression of the IL-8 gene (Hoffmann et al., 2002). Necessary for IL-8 gene expression is a nuclear factor-κB (NF-κB) dimer containing p65 (Rel-A). In resting cells, NF-κB is held inactive in the cytosol by an inhibitor of κB (IκB); however, NF-κB-activating stimuli facilitate phosphorylation of IκB by an IκB kinase (IKK) and subsequent degradation of IκB (Hoffmann et al., 2002). Liberated NF-κB dimers translocate to the nucleus where they interact with κB-containing enhancers and facilitate gene expression (Hoffmann et al., 2002).

Enteropathogenic *Escherichia coli* and EHEC rely on a type-III secretion system (T3SS) to dampen inflammatory signaling (Vallance and Finlay, 2000). Recently, several studies have demonstrated a role for the non-LEE encoded (Nle) effectors NleB, NleC, NleE, NleH1, and NleH2 in dampening NF-κB-mediated gene expression (Gao et al., 2009; Nadler et al., 2010; Newton et al., 2010; Royan et al., 2010; Yen et al., 2010; Baruch et al., 2011). NleB and NleE stabilize IκBα and thus limit p65 translocation to the nucleus, a mechanism which is shared by the T3SS effector OspZ from *Shigella flexneri* (Nadler et al., 2010; Newton et al., 2010). NleH1 and NleH2 both dampen NF-κB-mediated gene expression, but only NleH1 was shown to mediate this by decreasing nuclear abundance of ribosomal protein S3 (Gao et al., 2009; Royan et al., 2010). Four independent studies have recently shown that NleC is a metalloprotease that enzymatically degrades p65 (Yen et al., 2010; Baruch et al., 2011; Muehlen et al., 2011; Pearson et al., 2011). All four studies demonstrate that an *nleC* null (Δ*nleC*) strain of EPEC could not fully suppress NF-κB activity or IL-8 secretion to levels of wild-type (WT) EPEC. Ectopic expression of NleC could also suppress NF-κB activity and IL-8 secretion (Yen et al., 2010; Baruch et al., 2011; Muehlen et al., 2011; Pearson et al., 2011). All four studies identified a metalloprotease domain within NleC as essential for its function within host cells.

NleC is a zinc-dependent protease with a canonical HEXXH motif (Yen et al., 2010; Baruch et al., 2011; Muehlen et al., 2011). Although NleC is the first type-III secreted Zn-metalloprotease to be identified, other virulence factors have also been shown to degrade p65 to dampen the inflammatory response in the host. *Chlamydia trachomatis* and *C. pneumoniae* both utilize a T3SS to secrete chlamydial protease-like activity factor (CPAF) from chlamydial inclusions into the host cell cytosol (Christian et al., 2010). CPAF functions to degrade p65 and can dampen pro-inflammatory signaling in *Chlamydia* infected cells (Christian et al., 2010). Since *Chlamydia* species are obligate intracellular pathogens, it is not surprising that they encode virulence factors to dampen immune signaling. CPAF does not exclusively target p65 suggesting that NleC may also have other targets in the host cell.

The *nleC* gene is highly conserved between EPEC, EHEC, and *C. rodentium*, a natural A/E pathogen of mice, suggesting that it has a similar function in all three pathogens. The ability of NleC to dampen the release of IL-8 in culture would suggest that a *C. rodentium* Δ*nleC* strain would cause increased inflammation and colitis in murine hosts. This is an attractive theory; however, NleC has little impact on *C. rodentium* colonization and colon weight *in vivo* (Kelly et al., 2006). Use of a *C. rodentium nleC*/*nleE* double knockout would likely provide additional insight into the impact of NF-κB inhibition *in vivo*. NleC is the first p65-targeting metalloprotease to be identified in the effector arsenal of an extracellular pathogen. Recent insights into the function of NleC may aid in elucidating functions for other effector proteins in other pathogens. Understanding the role of effector proteins will further our understanding of their role in virulence and the pathogenic strategy of disease-causing bacteria.

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