The *Shigella Flexneri* Effector OspG Interferes with Innate Immune Responses by Targeting Ubiquitin-Conjugating Enzymes

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Bacteria of *Shigella* spp. are responsible for shigellosis in humans, a disease characterized by destruction of the colonic epithelium that is induced by the inflammatory response elicited by invasive bacteria. They use a type III secretion system injecting effector proteins into host cells to induce their entry into epithelial cells and triggers apoptosis in macrophages. We present evidence that the effector OspG is a protein kinase that binds various ubiquitinylated ubiquitin-conjugating enzymes (E2s) and blocks degradation of phospho-IκBα induced upon entry of bacteria into epithelial cells. Transfection experiments confirmed that OspG interferes with the NF-κB activation patway by preventing phospho- IκBα degradation, suggesting that OspG inactivates a component of the $SCF^{\beta\text{-}TrCP}$ ubiquitin ligase complex (E3) involved in phospho- IκBα ubiquitination. Upon infection of ileal loops in rabbits, the *ospG* mutant induced a stronger inflammatory response compared with the wild-type strain, indicating that OspG down-regulates the host innate response induced by invasive bacteria.

INTRODUCTION

The intestinal barrier is endowed with a balanced combination of detection and defence mechanisms to achieve both tolerance to commensal microorganisms and protection against invading microorganisms (1). Invasion by pathogens, both extra- and intra-cellular, is sensed by various signalling pathways and results in the establishment of an inflammatory response aimed at eradicating pathogens. NF-κB is a member of the Rel family of transcription factors that is involved in activation of a large number of genes in response to pathogens, stress signals and pro-inflammatory cytokines (2). NF-κB proteins are retained in the cytoplasm through their association with inhibitory proteins IκBs, including IκBα. A variety of signaling pathways activate IκB kinases (IKKs) to phosphorylate Ser-32 and Ser-36 of IκBα, leading to ubiquitination of phospho-IκBα and its subsequent degradation by the proteasome (3).

The ubiquitination pathway results in the covalent attachment of the 76-residue ubiquitin to target proteins in three sequential steps performed by one ubiquitin-activating enzyme (E1), a limited number of ubiquitin-conjugating enzymes (E2, Ubc) and a large number of ubiquitin-ligating enzymes (E3) respectively (4). Each E3 recognizes a set of substrates and cooperates with one or a few E2s. The E3 complex $SCF^{\beta\text{-}TrCP}$ promoting ubiquitination of phospho-IκBα consists of 5 proteins, the scaffold protein Cullin1, the adaptor protein Skp1, the RING domain protein Roc1, the F box protein β-TrCP that interacts with phospho-IκBα and the E2 UbcH5b (5).

Bacteria of *Shigella* spp. are responsible for shigellosis in humans, a disease characterized by the destruction of the colonic epithelium that is responsible for 1 million deaths per year (6). These bacteria use a type III secretion (TTS) system to enter epithelial cells and trigger apoptosis in macrophages (7). TTS systems are widely spread among Gram-negative pathogens and comprise (i) a secretion apparatus that spans the bacterial envelope, (ii) translocators that transit through the TTS apparatus and insert into the membrane of the host cell to form a pore, (iii) effectors that transit through the TTS apparatus and the translocator pore to be injected into the cell cytoplasm where they interfere with a variety of cellular functions, (iv) molecular chaperones and (v) specific transcription regulators (8). The *S. flexneri* TTS system is encoded by a 200-kb virulence plasmid and includes ≈ 20 potential effectors (9). The TTS apparatus is assembled during growth of *S. flexneri* in broth and is activated upon contact of bacteria with epithelial cells .

We present the functional analysis of the *S. flexneri* effector OspG, a 196-residue substrate of the TTS apparatus whose expression is regulated by secretion activity (9, 10). A two-hybrid screen in yeast identified ubiquitin conjugating enzymes (Ubc, E2) as potential partners of interaction of OspG and *in vitro* studies showed that OspG binds ubiquitinylated E2s (Ub-E2s) and is endowed with autophosphorylation activity. Transfection experiments indicated that OspG prevents degradation of phospho-IκBα and activation of an NF-κB regulated promoter. Characterization of the phenotype of an *ospG* mutant using both *in vitro* and *in vivo* models of infection confirmed that OspG is involved in the down-regulation of the host innate response induced by invasive bacteria (11).

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