Tyrosine kinases, drugs, and *Shigella flexneri* dissemination

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Shigella flexneri is an enteropathogenic bacterium responsible for approximately 100 million cases of severe dysentery each year. *S. flexneri* colonization of the human colonic epithelium is supported by direct spread from cell to cell, which relies on actin-based motility. We have recently uncovered that, in intestinal epithelial cells, *S. flexneri* actin-based motility is regulated by the Bruton's tyrosine kinase (Btk). Consequently, treatment with Ibrutinib, a specific Btk inhibitor currently used in the treatment of B-cell malignancies, effectively impaired *S. flexneri* spread from cell to cell. Thus, therapeutic intervention capitalizing on drugs interfering with host factors supporting the infection process may represent an effective alternative to treatments with antimicrobial compounds.

S. flexneri Actin-Based Motility Relies on N-WASP and the ARP2/3 Complex

Shigella flexneri is a Gram-negative intestinal pathogen, causing dysentery by invading the epithelium of the colon.¹⁻³ Once internalized in the intestinal cells, *S. flexneri* lyses the endocytic vacuole and gains access to the cytosolic compartment, where it displays actin-based motility. Seminal studies have revealed that *S. flexneri* actin-based motility relies on the polar expression of the bacterial factor IcsA, which mediates the recruitment of the host cell neural Wiskott-Aldrich Syndrome protein (N-WASP).⁴⁻⁸ The recruitment of N-WASP leads to the recruitment and activation the ARP2/3 complex, a major actin nucleator. Actin

polymerization at one bacterial pole generates forces that propel the bacteria throughout the cytosol of infected cells. As motile bacteria reach the cell periphery, they spread from cell to cell through formation of membrane protrusions that resolve into vacuoles in adjacent cells.⁹ This spreading process is central to the pathogenic properties of *S. flexneri* as the IcsA mutant defective in actin-based motility is essentially avirulent.

Regulation of N-WASP Activity

N-WASP is a multi-domain regulator of the actin cytoskeleton harboring an N-terminal small GTP-ase binding domain (GBD) domain that interacts with the small GTPase Cdc42, and a VCA domain that interacts with and promotes the actin nucleation activity of the ARP2/3 complex (Fig. 1A). The nucleation-promoting activity of N-WASP toward the ARP2/3 complex is regulated by auto-inhibition resulting from interaction of the GBD domain with the VCA domain (Fig. 1A).¹⁰ The interaction of activated Cdc42 with the GBD domain releases the auto-inhibited conformation, which allows for actin nucleation at the plasma membrane through interaction of the VCA domain with the ARP2/3 complex (Fig. 1A).^{11,12} In the context of filopodium formation and neurite extension, the activity of WASP/N-WASP family members is regulated by Src family kinases-dependent phosphorylation of tyrosine residue located in the GBD domain (Fig. 1B).^{13,14} This phosphorylation event is thought to contribute to the release of the auto-inhibited conformation by decreasing the affinity of the N-terminal GBD domain

Keywords: dissemination, spread from cell to cell, actin-based motility, *Shigella flexneri*, N-WASP, phosphorylation, Btk, tyrosine kinase, Ibrutinib

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Submitted: 05/01/2013; Revised: 07/02/2013;
Accepted: 09/17/2013; Published Online: 09/19/2013
<http://dx.doi.org/10.4161/gmic.26523>

Addendum to: Dragoi AM, Talman AM, Agaisse H. Bruton's tyrosine kinase regulates *Shigella flexneri* dissemination in HT-29 intestinal cells. *Infect Immun* 2013; 81:598–607; PMID:23230296; <http://dx.doi.org/10.1128/IAI.00853-12>

for the C-terminal VCA domain.^{11,15} In the context of *S. flexneri* actin-based motility, the release of the auto-inhibited conformation of N-WASP is mediated by interaction with IcsA (Fig. 1C).^{4,16} In addition, studies conducted in mouse embryonic fibroblasts revealed that phosphorylation of the N-terminal region of N-WASP, as mediated by the Src family kinases Abl1/2, increased the rate of bacteria displaying actin-based motility, presumably by decreasing the affinity of the GBD domain for the VCA domain and releasing the auto-inhibited conformation (Fig. 1B).¹⁷

A Role for Btk in *S. flexneri* Actin-Based Motility in Intestinal Cells

Numerous non-intestinal cell lines have been shown to support *Shigella* actin-based motility.¹⁷⁻¹⁹ However, studies examining the cellular determinants supporting *S. flexneri* dissemination in intestinal cells remain scarce. To address this knowledge gap, we have identified the HT-29 intestinal cell line as a viable system for modeling several aspects of *Shigella flexneri* infection.¹ In particular, we have demonstrated that, as expected from studies conducted in non-intestinal cells, *S. flexneri* actin-based motility relies on the bacterial factor IcsA and the host factors N-WASP in HT-29 cells. To further investigate the potential requirement for N-WASP phosphorylation in *S. flexneri* actin-based motility, we systematically depleted tyrosine kinases formerly implicated in N-WASP/WASP phosphorylation in various cellular systems. We found that Bruton's tyrosine kinase (Btk) depletion led to the most dramatic effect on *Shigella* actin based-motility in HT-29 cells. By contrast, individual depletion of Abl1 or Abl2 resulted in minor defects in *S. flexneri* actin-based motility, and depletion of Hck, Fyn, Lyn or Syk had no detectable effect. These results therefore established Btk as a major tyrosine kinase implicated in *S. flexneri* actin-based motility in intestinal cells. Given its notorious role in leukemia development, Btk was previously thought to be specifically expressed in B-cells.²⁰ However, our results based on cDNA sequencing and gene expression silencing

Figure 1. Regulation of N-WASP activity. Under non-stimulating conditions N-WASP is folded into an auto-inhibited conformation due to interaction between the N-terminal small GTP-ase binding domain (GBD) and the C-terminal VCA domain. (A) Binding of the GBD domain to the small GTP-ase Cdc42, (B) phosphorylation of the GBD domain by tyrosine kinases (TKs) or (C) binding to *S. flexneri* IcsA, release the VCA domain from the auto-inhibited conformation, which mediates actin assembly through recruitment of the ARP2/3 complex.

unambiguously demonstrate that Btk is expressed in the HT-29 intestinal cells, where it is required for *S. flexneri* actin-based motility and dissemination.

Btk Regulates N-WASP Phosphorylation in Intestinal Cells

To further understand the role of Btk in *S. flexneri* dissemination, we evaluated N-WASP localization at the bacterial pole in Btk-depleted cells (Fig. 2). We found that the defects observed in actin-based motility correlated with defects in N-WASP recruitment. Moreover, we determined that the level of N-WASP tyrosine phosphorylation was lower in Btk-depleted cells. Furthermore, we showed that a phosphorylation-defective version of N-WASP was not recruited to the bacterial pole as efficiently as wild-type N-WASP. Altogether these results suggest that in intestinal cells, Btk-mediated phosphorylation of N-WASP modulates N-WASP recruitment to the bacterial pole, which is required for initiating actin-based motility (Fig. 2). These results are in agreement with a model in which Btk-mediated tyrosine phosphorylation of the N-terminal region of N-WASP contributes to the release of the auto-inhibited conformation of N-WASP (Fig. 1B), which facilitates its

recruitment to the bacterial surface through interaction with IcsA (Fig. 1C). In conclusion, HT-29 cells provide a useful system to model intestinal infection with *Shigella flexneri*. However, we note that their transformation status may alter kinase-dependent signaling pathways involved in growth and survival, which may differ from the situation observed in non-transformed cells. Therefore, it will be critical to further validate the role of Btk in *Shigella* dissemination in non-transformed intestinal cells.

The Btk Inhibitor Ibrutinib Blocks *S. flexneri* Dissemination

The observation that *S. flexneri* actin-based motility relied on the tyrosine kinase Btk led us to hypothesize that specific inhibitors of Btk activity would negatively impact *S. flexneri* dissemination (Fig. 2). One such inhibitor, Ibrutinib,²¹ is used as a treatment for various B-cell malignancies in mono-therapy and in combination with other anticancer agents and chemotherapies. An ongoing phase II trial in patients with untreated, relapsed and unresponsive chronic lymphocytic leukemia (CLL) shows that the drug is well tolerated and highly effective.²² As hypothesized, we found that Ibrutinib significantly impaired the dissemination of *S. flexneri* in intestinal cells, revealing

Figure 2. Role of N-WASP phosphorylation in *S. flexneri* actin-based motility and dissemination. The nucleation-promoting factor N-WASP is recruited to the bacterial pole through interaction with the bacterial factor IcsA. (A) Phosphorylation of N-WASP by Btk promotes actin-based motility and formation of large infection foci. (B) Btk depletion (RNAi) or inhibition (Ibrutinib) decreases the number of bacteria recruiting N-WASP, which affects actin-based motility and leads to formation of small infection foci.

that, although traditionally used in leukemia therapy, this drug may also prove useful in the treatment of infectious diseases, such as *S. flexneri* infection. It is however important to consider the potential development of adverse effects. The most common adverse effects observed in patients treated with Ibrutinib, in the context of leukemia, are grade 1

or 2 toxicities, such as diarrhea, nausea, fatigue, while grade 3 or 4 toxicities, such as neutropenia, thrombocytopenia and anemia, were infrequent and dose independent.²³ Thus, Ibrutinib may be used for short periods of time, potentially in combination with standard antibiotics, in order to minimize adverse effects and optimize antibacterial therapy.

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Significance and Implications

On a global scale, the World Health Organization estimates that the bacteria causes 90 million serious cases of disease and leads to 108,000 deaths per year. Death from shigellosis occurs predominantly among people in developing countries, especially in children under age five. While shigellosis may be treated with antibiotics, many drug-resistant strains of *Shigella* are now emerging.^{24,25} Intense efforts have been made for the development of an effective vaccine against *Shigella*.²⁵ One of the main challenges in achieving an effective prophylactic tool against this pathogen is the need to protect against multiple serotype strains. Therefore, therapies that target the host response instead may be beneficial in the context of bacterial strains resistant to antibiotics or in the absence of vaccines with a broad spectrum. Our findings suggest that tyrosine kinases inhibitors such as Ibrutinib may be used for treating *S. flexneri* infection. This study therefore supports the general notion that a better understanding of the functional host/pathogen interface may suggest novel strategies for therapeutic interventions.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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