Review

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New insights into the crosstalk between *Shigella* and T lymphocytes

Wilmara Salgado-Pabón[1*], Christoph Konradt[1], Philippe J. Sansonetti[1,2], and Armelle Phalipon[1]**

[1] Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, INSERM U786, 28 Rue du Dr Roux, 75724 Paris Cedex 15, France
[2] Chaire de Microbiologie et Maladies Infectieuses, Collège de France, Paris, France

Subversion of host immune responses is the key infection strategy employed by most, if not all, human pathogens. Modulation of the host innate response by pathogens has been vastly documented. Yet, especially for bacterial infections, it was only recently that cells of the adaptive immune response were recognized as targets of bacterial weapons such as the type III secretion system (T3SS) and its effector proteins. In this review, we focus on the recent advances made in the understanding of how the enteroinvasive bacterium *Shigella flexneri* interferes with the host adaptive response by targeting T lymphocytes, especially their migration capacities.

Host immune response subversion

Pathogens have evolved strategies to avoid or resist host defense mechanisms. Although there is ample information on the mechanisms used by pathogenic bacteria to subvert the innate immune defense system, very little is known about how they affect the adaptive immune response. The key role of dendritic cells (DCs) as antigen-presenting cells (APCs) bridging innate and adaptive immunity renders them particularly attractive for pathogen targeting, as recently reviewed [1]. Besides DCs, B and T lymphocytes are two other critical cell types of adaptive immunity, acting as cellular effectors ensuring protective immunity. Induction of long-term protective immunity to acute infection is a complex process, with T cell activation being a key element for eliciting both efficient antibody- and cell-mediated immune responses. T cell activation relies on productive encounters between APCs bearing foreign antigens and cognate T lymphocytes, which are present at low frequencies. Motility of naïve T cells within lymph nodes (LNs), secondary lymphoid organs where pathogen-specific immune response are orchestrated, is of utmost importance for these encounters to occur rapidly and efficiently [2,3], and high motility coefficients have been proposed to play a key role in optimizing the effector response to pathogens [4]. Chemoattractant-mediated T cell motility is highly dependent on the actin cytoskeleton. In the context of *Shigella* infection, it is critical to realize that *Shigella* has the capacity to trigger massive actin cytoskeleton rearrangements, as demonstrated in intestinal epithelial cells [5,6]. Hence, if *Shigella* were to target the actin cytoskeleton in T lymphocytes, it could significantly affect T cell motility and subsequently the priming of specific immune responses. This was the premise of the studies discussed in this review, where evidence is provided that upon infection *Shigella* targets T lymphocytes and alters their migratory properties.

Host innate and adaptive immune responses elicited upon *Shigella* infection

Shigella is an enteroinvasive pathovar of *Escherichia coli* and the causal agent of the acute recto-colitis shigellosis, otherwise known as bacillary dysentery. Children under the age of 5 years living in endemic areas of developing countries are the main targets of the disease. Although the mortality rate has significantly decreased in the last two decades, morbidity remains unacceptably high and hence problematic. Unfortunately despite unrelenting efforts, there is no vaccine available to protect children against shigellosis [7–10]. Therefore, deciphering the bacterial strategies that impair host immune responses and render humans continuously susceptible to *Shigella* infection throughout childhood is critical in designing new rational-based vaccine candidates.

Potent and acute inflammation is the hallmark of the host innate immune response to *Shigella* infection, characterized by a rapid influx of polymorphonuclear cells (PMNs) leading to massive tissue destruction [11,12]. The pathogenesis of *Shigella* relies on the expression of a T3SS and its secreted effector proteins. A first wave of secreted effectors is required for cellular invasion and initiation of the inflammatory response upon bacterial targeting of resident macrophages and intestinal epithelial cells. A second wave of T3SS effectors targets mitogen-activated protein (MAP) kinases and the nuclear factor-κB (NF-κB) signaling pathway to control the inflammatory process and to promote bacterial survival [13–15]. Detailed molecular mechanisms involving T3SS virulence effectors

Corresponding author: Phalipon, A. (armelle.phalipon@pasteur.fr).
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[*] *Current address:* University of Iowa, Carver College of Medicine, Department of Microbiology, 51 Newton Road, Iowa City, IA 52242, USA.
[**] *Current address:* Department of Pathobiology, School of Veterinary Medicine, 380 South University Avenue, University of Pennsylvania, PA, USA.

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and their cellular protein targets have been recently reviewed [16–18]. The inflammatory response also includes the recruitment of innate T cells. The virulence protein ShiA encoded by a *Shigella* pathogenicity island located on the chromosome has been shown to control the level of inflammation induced upon infection, by limiting not only PMNs, but also innate T lymphocyte recruitment [19]. This is a relevant bacterial strategy considering that innate T lymphocytes, such as mucosal-associated invariant T (MAIT) cells, have been recently shown to be very effective in detecting and efficiently lysing *Shigella*-infected epithelial cells [20].

Whether T3SS effectors or other virulence proteins interfere with the priming of *Shigella*-specific immune responses is not known. However, indirect evidence suggests that effector-mediated targeting of adaptive immune cells is a possible mechanism of immune evasion. It has been known for decades that natural infection with *Shigella* fails to elicit long-lasting protective immunity, and several infection episodes are required to generate a short-term, mainly serotype-specific, antibody-mediated protection [21,22]. Interestingly, a similar phenomenon has been reported upon *Plasmodium falciparum* infection leading to malaria [23]. These are suggestive of pathogen strategies utilized to dampen the acquired immune response. Impairing pathogen-specific protection, and thus providing the possibility of infecting the same individual multiple times, is obviously an advantage for human-restricted pathogens such as *Shigella*. Furthermore, upon *Shigella* infection it is well established that high levels of DC, B cell, and T cell death occurs during infection, as observed in rectal biopsies of *Shigella*-infected individuals. The factors that lead to cell death during infection are still under investigation, but evidence suggests that acute inflammation plays a critical role. In a mouse model of infection mimicking acute inflammation occurring upon natural infection, the proinflammatory cytokine environment elicited in response to the bacterium is prone for the predominant induction of *Shigella*-specific $CD4^+$ T helper (Th) 17 cells (Th17 cells) [24]. In addition, even though *Shigella* is a facultative intracellular bacterium and despite its ability to actively secrete proteins into the host cytoplasm, another type of T lymphocytes, that is $CD8^+$ T lymphocytes that play a prominent role in generating adaptive immune responses to cytosolic microbial pathogens and their products, fail to be primed upon infection [24,25]. Finally, the proinflammatory property of *Shigella* also affects DCs, especially their recruitment, by decreasing the production of chemokine (C–C motif) ligand 20 (CCL20), which is required for DC migration to infected tissues [26].

Besides the effects of *Shigella*-induced acute inflammation on B and T lymphocytes, whether *Shigella* directly targets T or B cell function and the consequence of this was examined, focusing up to now on T lymphocytes. Direct interaction between *Shigella* and lymphocytes are likely to occur: (i) in the lymphoid follicles associated with the colonic mucosa after bacterial crossing of the intestinal barrier via M cells located within the lymphoid follicle-associated epithelium, (ii) in the lamina propria, the connective tissue underlying the intestinal epithelium, and/or (iii) the draining mesenteric LNs, which constitute the last host barrier for *Shigella*, thus preventing systemic dissemination [15]. Here, we present the *in vitro* and *in vivo* approaches used to assess the *Shigella*–T cell interactions, and discuss the evidence for a role of the T3SS in modulating T cell motility and dynamics. Strategies evolved by bacterial pathogens to dampen T cell functions in general are also mentioned.

Outcomes of *Shigella*–T lymphocyte crosstalk: inhibition of $CD4^+$ T cell migration *in vitro*

First, to simplify the complexity of the *in vivo* settings, whether there are direct interactions of *Shigella* with T cells was addressed *in vitro*. By using a classical *in vitro* model of cell migration towards a chemoattractant, T cell migration was severely impaired in activated human $CD4^+$ T cells infected with invasive *Shigella* but not with a *Shigella* T3SS mutant. Surprisingly, injection of the T3SS effectors of *Shigella* into activated human $CD4^+$ T cells with no subsequent bacterial invasion also caused impaired migration. Interestingly, *Shigella* only impairs the migration of activated T cells. Indeed, unactivated cells infected with invasive *Shigella* migrate at similar levels as uninfected cells. Hence, *Shigella* preferentially targets activated T lymphocytes [27].

Directional migration of T lymphocytes towards a chemoattractant relies at an early stage on a protein family of membrane cytoskeleton crosslinkers called ERM (ezrin, radixin, and meosin). ERMs are implicated in cell cortex organization and provide a conformationally regulated connection from the cortical actin cytoskeleton to the plasma membrane. The rapid conversion of activated ERMs (phosphorylated) to the inactivated (dephosphorylated) conformation is critical for its function and depends on the concentration of phosphatidylinositol 4,5-bisphosphate (PIP_2) at the plasma membrane. Interestingly, PIP_2 is a target for the *Shigella* T3SS effector invasion plasmid gene for Ipg (Ipg)D, a phosphoinositide 4-phosphatase that hydrolyses PIP_2 into phosphatidylinositol 5-monophosphate (PI_5P), decreasing the PIP_2 pool at the plasma membrane and provoking a massive and sustained dephosphorylation of ERMs. *In vitro* studies with an IpgD deletion strain and its complemented counterpart provided evidence that IpgD is responsible for the $CD4^+$ T cell migration deficiency observed in *Shigella*-infected cells [27]. It is therefore proposed that the enzymatic activity of IpgD on PIP_2 affects posteriorization of the plasma membrane, known as polar-cap formation, essential for directional migration of T lymphocytes upon chemokine stimulation, with possible catastrophic consequences for the development of *Shigella*-specific adaptive immune responses (Figure 1).

Phosphatidylinositol (PI) metabolism plays a key role in the regulation of receptor-mediated signal transduction, actin remodeling, and membrane trafficking in eukaryotic cells [28–30]. Thus, it is not surprising that several intracellular bacterial pathogens modulate and exploit PI levels, directly or indirectly, to ensure their survival and efficient intracellular replication. In addition to several previous reports, a new recent example is the HIV Tat protein that binds with a high affinity to PIP_2, resulting in the perturbation of the PIP_2-mediated recruitment of