Shigella Targets T Cells

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Using a syringe-like device, *Shigella* delivers an array of virulence factors into host cells to facilitate bacterial colonization and disable the host's innate immune defense. In this issue of *Cell Host & Microbe*, Konradt and colleagues (Konradt et al., 2011) show that *Shigella* also subverts adaptive immunity by targeting T cells through a mechanism involving PIP_2 breakdown.

Pathogens have evolved multiple, often redundant and overlapping mechanisms to evade the cellular and humoral innate immune defenses that the host deploys upon sensing infection. For bacterial pathogens, these mechanisms include inhibition of phagocytosis by capsular polysaccharide, recruitment of complement regulators to the bacterial surface, and paralysis of phagocytes by secreted or intracellularly delivered toxins. Less well documented is the ability of bacterial pathogens to prevent the generation of an adaptive immune response or evade the specific adaptive immune defenses once these have developed. In the last few years, it has been shown that toxins from diverse bacteria such as *Helicobacter pylori, Bordetella pertussis*, and *Bacillus anthracis* are capable of entering $CD4^+$ T cells and incapacitating them through different molecular mechanisms (Baldari et al., 2005; Rossi Paccani et al., 2008; Tournier et al., 2009). As a result of these attacks, the generation of effector helper T cells is impaired, resulting in the failure of T cells to potentiate the bactericidal activity of phagocytes and to provide help to antigen-specific B cells to develop into high-affinity antibody-producing plasma cells.

Shigella, the causative agent of bacillary dysentery, very effectively evades the adaptive immune response. In fact, a protective immune response is only observed after multiple infections and is relatively short-lived. This is in part explained by the bacterium's ability to invade epithelial cells and to spread directly from cell to cell, thus avoiding immune detection. In addition, *Shigella* downregulates chemokine expression in infected epithelial cells, resulting in reduced recruitment of dendritic cells (Phalipon and Sansonetti, 2007). Konradt and collegues (Konradt et al., 2011) now describe another mechanism of adaptive immune repression by *Shigella* that involves a toxin-mediated attack on $CD4^+$ T cells. The difference with *Shigella* is that the toxin involved is not a secreted soluble toxin but an effector protein that is injected directly into the cytoplasm of the T cell through a type III secretion system (TTSS), a syringe-like structure that permits the delivery of several effector proteins directly from the bacterial cytoplasm to the target cell cytoplasm.

The paper describes several interesting findings. *Shigella* is shown to invade activated but not quiescent $CD4^+$ T cells. The reason for this selectivity is not known, although the authors hypothesize a potential role of molecules upregulated during T cell activation, such as CD44 or $\alpha5\beta1$ integrins. Invasion is dependent on a functional TTSS. The *Shigella* TTSS is a major virulence factor that is necessary for epithelial cell invasion and induction of inflammation. In addition, *Shigella* uses this system to inject effector proteins into phagocytes, resulting in actin disorganization, suppression of gene transcription, and ultimately cell death (Ashida et al., 2011). However, cell invasion is not necessary for the effect on T cells, as it is shown that the TTSS on the surface of the bacteria can engage the plasma membrane of the cell from the outside to deliver the effector proteins to the cytoplasm immediately upon contact with the cell. The end result is that the T cells become refractory to chemokine-dependent migration. Chemotaxis is crucially required for $CD4^+$ T cells which have differentiated to effectors in secondary lymphoid organs to reach their sites of action (the T:B cell zone in lymphoid tissues to provide help to activated B cells, the infected tissue to provide help to phagocytes). Hence, by impairing chemotaxis, *Shigella* effectively suppresses the adaptive immune response.

Using *Shigella* mutants lacking individual TTSS effector proteins, IpgD, a phosphatidylinositol 4-phosphatase that hydrolyses phosphatidylinositol 4,5-biphosphate (PIP_2), was identified as being necessary and sufficient to impair the chemokine-induced migration of T cells. Interestingly, IpgD was not necessary for cell invasion; however, on injection, IpgD was shown to rapidly deplete the plasma membrane pool of PIP_2, resulting in reduced phosphorylation and inactivation of ezrin, a member of the ezrin, radixin, and moesin (ERM) protein family (see Figure 1). These proteins connect cortical F actin to the plasma membrane, thereby contributing to the reorganization of actin cytoskeleton that is necessary for cell polarization and migration (Fehon et al., 2010). Hence, by modulating the levels of PIP_2 through IpgD, *Shigella* has evolved an effective mechanism to target the actin cytoskeleton. Interestingly, other *Shigella* TTSS effectors also attack the actin cytoskeleton, and remodeling of the actin cytoskeleton is necessary for invasion of epithelial cells (Ogawa et al., 2008). It is intriguing that these effectors do not seem to have any effect on T cell chemotaxis.

Inhibition of T cell chemotaxis appears to be a winning strategy to impair the development or function of helper T cells, as witnessed by its widespread use by both bacterial (e.g., *B. pertussis*, *B. anthracis*) and viral (e.g., HIV-1, HCV) pathogens. Interestingly, modulation of PIP_2 is emerging as a common target of

virulence factors of unrelated pathogens (e.g., *Salmonella* SopB, HIV-1 Tat), and this report by Konradt et al. (2011) further underlines this point, providing evidence that the response to the depletion in PIP_2 is mediated by inactivation of ERM proteins.

Of note, the activity of PIP_2 is not limited to promoting the reorganization of the actin cytoskeleton (Parry et al., 2007). PIP_2 regulates a number of cellular processes by assisting the recruitment and stabilization to the cytosolic face of the plasma membrane of a number of signaling mediators containing pleckstrin homology (PH) domains, a central one being the kinase Akt/PKB, which is essential for survival signaling and also promotes the polarization of Th1 cells. It may therefore be expected that, when injected into the cytosol of activated T cells, IpgD may have pleiotropic functions beyond F actin remodeling and chemotaxis.

With the identification of T cells as targets of *Shigella*, Konradt et al. (2011) not only provide a convincing explanation as to why adaptive immunity is short lived in the context of *Shigella* infection but also open a new area of investigation. The TTSS has been amply documented as a powerful device to deliver a variety of effectors to the cytosol of the host cell, and IpgD might represent only the tip of the iceberg. It is conceivable that other effectors are coinjected with IpgD into T cells. These include GEF mimicks, a protein tyrosine kinase, a phosphothreonine lyase, and ubiquitin ligases (Schroeder and Hilbi, 2008), which may subvert the signaling pathways controlling important cellular processes.

On a more general note, the studies of the interactions of bacterial pathogens with the host immune system have been largely focused on innate immunity or, at most, on the link between innate and adaptive immunity. Adaptive immunity is finally emerging as a central target of the immune evasion strategies evolved by bacteria. In the case of *Shigella*, the bacterium can cross the intestinal epithelium via M cells localized in the mucosa-associated lymphoid tissue, where it can encounter naive T cells. Furthermore, after disrupting the epithelial barrier, *Shigella* can reach the lamina propria, where it can encounter activated T cells, as directly shown in the Konradt et al. (2011) report, where invasion of T cells in vivo after crossing the epithelial barrier is documented using a rabbit ileal loop ligation model. Hence, to achieve a comprehensive understanding of how pathogens suppress or subvert the immune response, adaptive immune cells must be put into the picture.

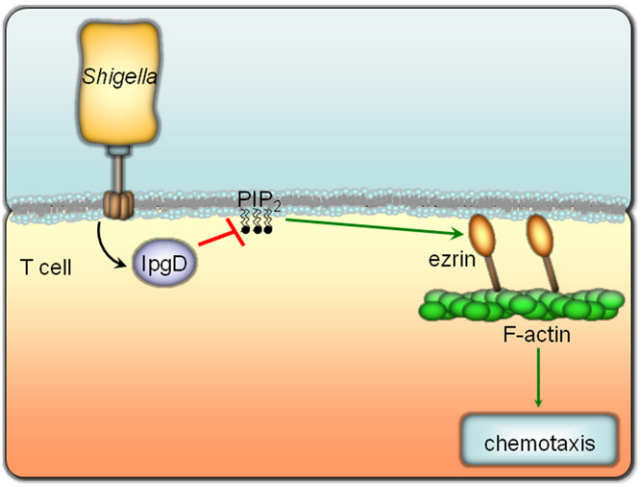

Figure 1. Impairment of T Cell Chemotaxis by *Shigella*
Schematic representation of the mechanism by which *Shigella* IpgD impairs T cell migration. Shigella injects the lipid phophatase IpgD into the cytosol of activated $CD4^+$ T cells, causing a reduction in the cortical pool of PIP_2 and inactivation of the membrane-cytoskeleton linker ezrin, which results in defective chemotaxis.

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