

Immune Response to *Salmonella*: Location, Location, Location?

Minireview

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Successful immunity against *Salmonella* infections is dependent on the generation of CD4⁺ T helper cells and to a lesser extent on antibody production and CD8⁺ T cells. The cells within the lymphatic tissue of the gut are likely to be central for the orchestration of a proper and rapid response. The anatomical restriction of the pathogen may also determine the distribution of effector cells. In this issue of *Immunity*, McSorley et al. address both of these processes using identifiable CD4 T cells that are specific for *Salmonella typhimurium*. Such cells localize to the Peyer's patches of the small intestine when the bacteria are delivered orally.

Salmonella enterica are enteropathogenic bacteria that can cause a variety of syndromes ranging from common food poisoning to the sometimes life-threatening typhoid fever. The type of disease caused by these bacteria depends not only on the serovar (e.g., *S. typhimurium*, *S. typhi*, *S. enteritidis*, etc.) of the infecting bacteria but also on the species and immunological status of the infected hosts (Ohl and Miller, 2001). Despite the widespread nature of this pathogen, it is often overlooked that the overwhelming majority of *Salmonella* infections are subclinical. Indeed, *Salmonella* has evolved a very complex functional interphase with its hosts, the product of evolution throughout its long-standing association with vertebrate animals (Galán, 2001). This functional interphase, which is beginning to be understood in great detail at the molecular level, is best characterized by its refinement rather than by its potential for harm. It is now clear that *Salmonella* engages its host in remarkable biochemical interactions that lead to a series of well-coordinated cellular responses. These cellular responses allow the bacteria to reach privileged niches, where they can replicate and complete their life cycle within the host.

After oral infection, usually through contaminated food or water, *Salmonella* can survive exposure to the low pH of the stomach and arrive in the intestine where it can penetrate the epithelial layer (Galán and Sansonetti, 1996). This phase of the *Salmonella* life cycle is largely dependent on the function of a specialized protein delivery system (termed type III), which "injects" bacterial proteins into the host cell with the capacity to specifically stimulate or inhibit cellular responses. A subset of these proteins stimulates the activity of the Rho family GTPases Cdc42 and Rac by either directly catalyzing nucleotide exchange or by stimulating endogenous exchange factors (Galán, 2001). As a result of this activation, intestinal epithelial cells undergo profuse actin cy-

toskeleton rearrangements leading to the uptake of the bacteria into a membrane-bound compartment. In addition, activation of Cdc42 and Rac leads to the reprogramming of gene expression in the infected intestinal epithelial cells, ultimately resulting in the production of a variety of proinflammatory cytokines. Remarkably, these bacteria are actively engaged in the reversion of the cellular responses stimulated by the initial interaction. Through the injection of a GTPase-activating protein (GAP) for Cdc42 and Rac, *Salmonella* effectively halts the responses resulting from the activation of these GTPases, contributing to the recovery of the host cell and thus preserving the integrity of its replicative niche (Fu and Galán, 1999). These complex interactions that occur during the initial stages of infection are not only an eloquent example of the sophistication of the biology of these bacteria but also a demonstration that these microorganisms are active participants in the host/pathogen interaction.

The actual primary site at which *Salmonella* breaches the intestinal epithelium has not been precisely defined, and it is a matter of some controversy (Galán and Sansonetti, 1996). It is nevertheless clear that the site of entry is dependent on both the serovar of the infecting *Salmonella* as well as the species of the infected host. It is often cited in textbooks that in humans the distal part of the small bowel is the primary site of *Salmonella* infection. However, there is remarkably little direct evidence to support this assertion. In other animals, the involvement of other segments of the intestinal tract is well documented. There is also some controversy regarding the role of Peyer's patches as a site of entry for *Salmonella*. The follicle-associated epithelium (FAE) overlying the Peyer's patches contains specialized cells, designated M cells ("M" for microfolds or membranous cells). These cells have increased pinocytotic activity and deliver microbes and antigen via transepithelial vesicular transport from the gut lumen to macrophages and lymphocytes residing below the epithelium. Although it is well demonstrated that at least in some animal models *Salmonella* can replicate in Peyer's patches (Jones et al., 1994), it is also well documented that *Salmonella* can breach the intestinal barrier through the cells of the columnar epithelium (Takeuchi, 1967). In fact, it has been argued that since these cells vastly outnumber intestinal M cells, they may well constitute the main port of entry for *Salmonella*. Once the intestinal barrier has been breached, the fate of *Salmonella* varies widely depending on its serovar or the species of the infected host. In most cases, *Salmonella* remains localized to the intestinal epithelium and the gut-associated lymphoid tissues. In rare cases, *Salmonella* becomes systemic and invades deeper tissues.

The innate immune system plays an essential role in the early responses to *Salmonella* and in most subclinical infections may be enough to control progression to disease (Lalmanach and Lantier, 1999; Mäkelä and Hormaeche, 1997). The importance of macrophages and polymorphonuclear neutrophils in the early responses to *Salmonella* is well documented (O'Brien et al., 1979;

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Vassiloyanakopoulos et al., 1998). The stimulation of proinflammatory cytokine production (e.g., TNF- α , IL-1, IL-6, IL-8, IL-12, etc.) by agonists of Toll receptors (e.g., LPS, lipoprotein, flagellin, etc.) and by specific bacterial effectors delivered by *Salmonella* through its type III secretion system is also likely to be an important component of this phase of the defense response (Galán, 2001; Lalmanach and Lantier, 1999). Although the innate immune system is the primary line of defense against *Salmonella* infections, it is clear that the acquired immune system is important for clearing the infection as well as for providing effective protection to subsequent challenge with related *Salmonella* strains. Most of what is known about the immune response to *Salmonella* comes from studies using a mouse model of infection (Mittrucker and Kaufmann, 2000). Although this model exhibits the obvious advantage of the ease with which mice can be genetically manipulated, it has the limitation that it does not faithfully replicate the natural infection. For example, in humans, *Salmonella typhimurium* usually causes a self-limiting diarrhea (food poisoning). In contrast, in the mouse, *Salmonella typhimurium* causes a systemic infection that resembles typhoid fever in humans, a disease caused by *S. typhi*, a significantly different serovar. In addition, while wild-type (Ity^r) mice are relatively resistant to *Salmonella typhimurium*, the most often utilized Ity^s mice are highly susceptible to *S. typhimurium* infection (O'Brien et al., 1980). These mice are defective in the natural-resistance-macrophage-associated protein 1 (Nramp-1) (Forbes and Gros, 2001). Because of the increased susceptibility of these mice, most studies have been carried out using this genetic background, which certainly complicates the extrapolation of these findings to the understanding of the immune response to *Salmonella* during natural infection. Furthermore, some studies have been conducted using unnatural routes of infection (e.g., intravenous or intraperitoneal) or using attenuated strains of *S. typhimurium*, which has led to inconsistent results. Despite these complications and caveats, experiments conducted with specific gene-deficient mice have been very valuable in contributing to the understanding of the immune response to *Salmonella* (Mittrucker and Kaufmann, 2000).

There is general consensus about the importance of T cells in acquired immunity to *Salmonella* (Mäkelä and Hormaeche, 1997; Mittrucker and Kaufmann, 2000). Nude mice and mice deficient in $\alpha\beta$ T cells are more susceptible to *Salmonella* infections. However, the relative contribution of the different T cell subsets has been a matter of some controversy. In most cases, CD4⁺ T cells have been shown to be more important than CD8⁺ T cells, particularly in adoptive transfer experiments (Mastroeni et al., 1992; Nauciel, 1990). Consistent with an important role for CD4⁺ cells, MHC class II-deficient mice showed increased susceptibility to *Salmonella* (Hess et al., 1996). However, evidence indicates that CD8⁺ cells also contribute to the protective immune response to *Salmonella* since in at least one report, β 2m-deficient mice were shown to be more susceptible to *Salmonella* infection (Lo et al., 1999; Mastroeni et al., 1992; Nauciel, 1990). Nevertheless, it is clear that CD4⁺ T cells play a more prominent role in immunity to *Salmonella*. CD4⁺ helper T cells (T_H) are divided into two types

depending on the profile of cytokines they secrete. T_H1 cells produce IFN- γ and TNF- α and activate cellular immunity and inflammation, while T_H2 cells produce IL-4, IL-5, and IL-13 and induce B cell activation and differentiation. A number of studies have shown that *Salmonella* infection results in the induction of a T_H1 response (Pie et al., 1997; Thatte et al., 1993). It has also been shown that administration of exogenous IFN- γ to mice has bacteriostatic effects and that neutralization of endogenously produced IFN- γ by specific antibodies increases the mortality of mice infected with *S. typhimurium* (Matsumura et al., 1990; Ramarathinam et al., 1991). These results, in combination with the observation that mice deficient in IFN- γ receptors are highly susceptible to *Salmonella* infection (Hess et al., 1996), further support a crucial role for CD4⁺ T_H1 cells in *Salmonella* protection.

In addition to T cell-mediated immunity, the production of antibody has been proposed to be important in mediating immunity to *Salmonella*, and there have been many studies that have presented evidence supporting a role for B cells and antibody production in conferring protection (Mäkelä and Hormaeche, 1997; Mastroeni et al., 1993, 2000). *Salmonella* infections result in potent antibody responses, particularly to LPS. However, the contribution of antibodies to *Salmonella* immunity has been controversial given conflicting findings after induction of passive immunity by transfer of serum to naive mice. It has been recently shown that mice with a targeted disruption of the Ig μ gene (Igh-6^{-/-}), which are deficient in B cells, showed increased susceptibility to *Salmonella* infection and an inability to mount a strong convalescent immune response (Mastroeni et al., 2000). Therefore, it appears that B cells may influence the longevity or quality of the T cell-mediated responses.

Despite these significant advancements in the understanding of *Salmonella* acquired immunity, it is still not known where T cells first encounter antigen-presenting cells bearing MHC/*Salmonella* antigen peptide complexes. Dendritic cells located in Peyer's patches and other areas of the intestinal tract are the prime candidates to be responsible for first sampling *Salmonella* antigens, since these bacteria are most often restricted to this anatomical site during natural infection (with the exception of the serovars capable of causing typhoid fever such as *S. typhi*) (Yrlid et al., 2001). In fact, studies have suggested that dendritic cells are capable of taking up *Salmonella* directly from the lumen of the intestinal tract (Rescigno et al., 2001). Some studies have shown rapid translocation of *S. typhimurium* to deeper tissues (Vazquez-Torres et al., 1999), suggesting that perhaps antigen-presenting cells located at sites distant from the intestinal epithelium could come in contact with *Salmonella* antigens very early during infection. However, these studies have been carried out in strains of mice that are highly susceptible to *Salmonella* infections due to the deficiency in the macrophage-associated protein Nramp. Such high susceptibility introduces a major caveat in the interpretation of these experiments, since the pathogenesis of *Salmonella* is undoubtedly very different in these mice and the anatomic restrictions in the generation of the immune response may be significantly affected. In a study investigating the role of T_H1 cells in *S. typhimurium* immunity, orally infected mice showed T cell responses in the Peyer's patches (George, 1996).

However, parenterally inoculated mice did not show such a response. Only when these mice were rechallenged were T cell responses detected in Peyer's patches. From these results, it was concluded that *Salmonella* could elicit a T_H1 response in Peyer's patches and mesenteric lymph nodes and that priming of T cells could occur at other nonmucosal sites, but only orally administered *Salmonella* could direct T cell activity to the lymphatic tissue of the gut. These studies also underscored the importance of utilizing the natural route of infection in the study of the immune response to an intestinal pathogen.

In this issue of *Immunity*, McSorley et al. report the very intriguing observation that *Salmonella typhimurium* induces a very localized mucosal $CD4^+$ T cell response after oral inoculation despite the fact that the experimental conditions utilized were such that led to a disseminated infection (McSorley et al., 2002). An adoptive transfer system was used to monitor the distribution and fate of flagellin-specific $CD4^+$ T cells after *S. typhimurium* infection of highly susceptible N-ramp-deficient mice. They observed a remarkably rapid activation (within 3 hr after oral infection) of $CD4^+$ T cells in the Peyer's patches of orally inoculated mice. Although this rapid activation kinetics may be in part due to the rather massive bacterial dose used in these studies (~50,000 LD50), this observation does underscore the rapidity and efficiency with which *Salmonella* is capable of engaging the immune system. These results also underscore the often overlooked fact that bacterial pathogens, unlike inert antigens, are active participants in the induction of host responses and therefore have the potential to modulate (negatively or positively) the kinetics of the immune response. This rapid activation also suggests that dendritic cells in T cell areas of Peyer's patches must be able to rapidly present antigen to nearby T cells without migrating to the mesenteric lymph nodes, as has been traditionally proposed. McSorley et al. also observed a strict anatomical compartmentalization of activated flagellin-specific $CD4^+$ cells. Activated $CD4^+$ cells were observed almost exclusively in Peyer's patches and mesenteric lymph nodes, and no clonal expansion was observed in other systemic anatomical sites including spleen and other lymphoid organs. This is indeed a surprising finding since there was significant bacterial load at these systemic sites. McSorley et al. hypothesize that their findings may indicate that there is a rather strict anatomical compartmentalization between APCs and T cells, creating an anatomical barrier that prevents T cell activation. Although this hypothesis may well be correct, it is important to point out that there is an alternative explanation for these findings. Although the bacterial load in systemic organs was significant, this does not necessarily mean that the antigenic load (i.e., levels of flagellin) was equivalent. The authors state that APC from spleens were apparently able to stimulate a flagellin-specific T cell in vitro. However, it is possible that the number of flagellin-loaded APCs was sufficient to obtain a read out on a sensitive in vitro assay but was not enough to significantly expand the adoptively transferred T cells to a level detectable by the experimental methods used. It is very well established that bacterial pathogens rapidly reprogram gene expression during their infection cycle (Mahan et al.,

2000). *Salmonella typhimurium*, for example, expresses different sets of genes for the initial interaction with the intestinal epithelium than those utilized to cause systemic infection (Galán, 2001). Although it has not been specifically investigated, it is entirely possible or even likely that flagellin is not expressed by *Salmonella* when it reaches the spleen. Indeed, it has been shown that flagellar gene expression is negatively regulated by the PhoP/PhoQ bacterial two-component regulatory system (Adams et al., 2001). Since this regulatory system plays a prominent role during systemic infection, it is indeed very likely that genes negatively regulated by PhoP/PhoQ are turned off during this phase of the infection (Groisman, 2001). This is the case, for example, for the genes that encode the type III secretion system that *Salmonella* utilizes during its initial interaction with the intestinal epithelium (Galán, 2001). This system, which is coregulated with the flagellar system (Eichelberg and Galán, 2000) and also negatively regulated by PhoP/PhoQ (Pegues et al., 1995), is switched off during the systemic phase of infection. Therefore, it is possible that the lack of expansion of flagellin-specific $CD4^+$ cells in the spleen may simply be the consequence of lack of flagellin expression by the systemically localized bacteria. Experiments with bacteria constitutively expressing the test antigen will be necessary to rule out this possibility. The authors also argue that in the spleen, *Salmonella* and the adoptively transferred T cells may reside in different compartments, which may contribute to the anatomical separation between relevant APCs and $CD4^+$ T cells and the lack of expansion of those cells. However, it has been previously shown that *Salmonella* is indeed capable of stimulating *Salmonella*-specific $CD4^+$ T cells in the spleens of infected mice when parenterally administered (George, 1996). Therefore, it would have to be argued that *Salmonella* ends up in a different spleen area when parenterally administered, which is unlikely based on what is known about its systemic dissemination through the bloodstream. A significant difference in the experimental set up that resulted in expansion of $CD4^+$ T cells in the spleen upon *Salmonella* infection is that the indicator antigens used in those studies (bacterial extracts) were certainly expressed by *Salmonella* when localized in the spleen. Despite this caveat (and perhaps because of it), McSorley et al. convincingly show that $CD4^+$ cells stimulated at mucosal sites such as Peyer's patches migrate inefficiently to systemic sites (McSorley et al., 2002). In contrast, when adoptively transferred mice were immunized intravenously with LPS and flagellin peptide, $CD4^+$ flagellin-specific T cells were detected throughout all compartments, including mesenteric lymph nodes, liver, and lamina propria. McSorley et al.'s observations contrast those reported by Huleatt et al., who showed that oral and parenteral immunization of mice with *Listeria monocytogenes* resulted in an essentially identical distribution of bacterial peptide-specific $CD8^+$ T cells throughout the spleen, intestinal epithelium, as well as the lamina propria (Huleatt et al., 2001). However, since mice are not permissive for *Listeria monocytogenes* replication in the intestinal epithelium, it is likely that this experimental set up was simply not capable of resolving potential differences in the immune response after oral or parental inoculation.

Therefore, it appears that the location of the infection may determine the characteristics of the subsequent effector response to microbial infections. Future studies will have to rigorously consider the role of the anatomical restriction of the replicating pathogen, the levels of antigen expression in different compartments, as well as the active role that pathogens may play in modulating the immune response.

Selected Reading

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