Tracking the dynamics of T-cell activation in response to *Salmonella* infection

Rajesh Ravindran and Stephen J. McSorley

Department of Medicine, Division of Immunology, University of Connecticut Health Center, Farmington, CT, USA

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Correspondence: Dr S. J. McSorley, Department of Medicine, Division of Immunology, University of Connecticut Health Center, Farmington, CT 06030, USA.
Email: mcsorley@uchc.edu
Senior author: Stephen J. McSorley

Summary

Despite the current availability of *Salmonella* vaccines, typhoid fever remains a significant public health problem in developing countries. A greater understanding of T-cell activation and the development of immunological memory during *Salmonella* infection should lead to the development of more effective prophylactic intervention. Here, we review recent literature on the initiation, expansion and memory development of T-cell responses using the mouse model of typhoid. We pay particular attention to strategies for tracking T-cell responses *in vivo* and *ex vivo*, and suggest models to integrate some these studies.

Keywords: T cell; *Salmonella*; bacteria; memory; vaccination

Introduction

Salmonella enterica are Gram-negative bacteria that infect humans and animals, causing a spectrum of disease ranging from systemic infection to gastroenteritis, depending on the particular bacterial serovar and the host species infected.[1] Typhoid fever is a systemic disease caused by *Salmonella enterica*, serovar *typhi*, a highly invasive enteric pathogen found almost exclusively in developing counties. According to World Health Organization estimates, the annual global incidence of typhoid fever is around 16 million cases per year, and accounts for 600 000 deaths.

Salmonella enterica serovar *typhimurium* (hereafter referred to as *S. typhimurium*) infection of susceptible mouse strains causes an invasive systemic disease that is similar in many respects to typhoid fever.[2] This model is widely accepted as the best experimental system for studying human typhoid fever and has proved extremely valuable in uncovering mechanisms of innate and acquired immune resistance to intracellular pathogens.

Immune responses to *Salmonella* in mice

Host defence against *S. typhimurium* infection requires significant contributions from both the innate and acquired arms of the immune system.[1,3] The initial stages of infection are characterized by an innate immune response triggered by host recognition of several microbial structures[4] including pathogen-associated molecular patterns such as flagellin, lipopolysaccaride (LPS), and lipoproteins. Each of these bacterial products can induce the production of inflammatory cytokines that are likely to contribute to the initial control of *Salmonella* infection.[5–7]

Salmonella infection also induces antigen-specific CD4 T-cell, CD8 T-cell, and B-cell responses, all of which can contribute to protective immunity. Many immunodeficient mouse strains are unable to control the *in vivo* replication of attenuated *Salmonella* strains, providing a reasonable model for determining the contribution of different cell types to the primary immune response. For example, nude, T-cell receptor αβ-deficient, and major histocompatibility complex (MHC) class-II deficient, mice all succumb to infection with strains of attenuated *Salmonella* that are normally eradicated in wild-type mice.[8–10] In marked contrast, mice lacking MHC class-I restricted T cells, display no difference, or only a mild defect in the resolution of primary infection with attenuated *Salmonella*.[9,11] Similarly, several groups have reported that mice lacking B cells are able to control primary infection with attenuated *Salmonella* in a manner similar to wild-type controls.[12–14] At face value, these studies would suggest that CD4 T cells are critical for resistance to *Salmonella*, and that other lymphocyte populations are relatively unimportant. However, these immunodeficient mouse models do not paint a true picture of the

complexity of the host immune response to *Salmonella*. In fact, the true requirements for immunity to murine typhoid are apparent when re-infecting vaccinated mice with virulent strains of *Salmonella*.

It has been known for some time that vaccination of susceptible mice with attenuated strains of *Salmonella* provides robust immunity to re-challenge with virulent *Salmonella*.[15] However, it has proved difficult to transfer this protective immunity to naïve recipients unless T cells and serum antibodies are both transferred.[16] Similarly, MHC class-I-deficient and B-cell deficient mice resolve initial infection with vaccine strains of *Salmonella* but, unlike wild-type mice, this provides no protection against re-challenge with virulent *Salmonella*.[11–14] Thus, a greater role for CD8 T cells and B cells is uncovered using a model of re-challenge with virulent *Salmonella* than is observed when infecting immunodeficient mice with attenuated bacteria. Therefore, despite the simplicity of the model system, it seems unlikely that infection of immunodeficient mice with slow-growing *Salmonella* provides an accurate model of the immune response to typhoid fever, and results should be interpreted with some caution. Together, the available data using the mouse model point to a central role for CD4 T cells in the development of protective immunity to *Salmonella*, and an extremely important contributory role for both CD8 T cells and B cells. Indeed, these data nicely parallel work in human typhoid infection, where *Salmonella*-specific CD8 T-cell and B-cell responses can be detected following exposure to attenuated *Salmonella*, and are likely to play an important role in mediating protective immunity.[17–21]

The involvement of T-cell responses in mediating protective immunity to *Salmonella* infection has been apparent for decades. However, a detailed examination of *Salmonella*-specific T-cell activation has been lacking, because in large part of restrictions in the tools that are available in this model. The limited number of defined class-I and class-II *Salmonella* epitopes makes any attempt to examine *Salmonella*-specific T cells particularly challenging.[22] However, a number of recent studies have succeeded in examining the *in vivo* activation and expansion of CD4 T during *Salmonella* infection cells by using T-cell receptor (TCR) transgenic adoptive transfer systems.[23] Of particular relevance is that some of these studies have examined *Salmonella*-specific T-cell activation in the intestine, the most likely physiological site of initial activation.

Initial activation of naïve *Salmonella*-specific T cells

TCR transgenic adoptive transfer systems are an experimental methodology that simply raises the frequency of circulating antigen-specific T cells above the level of detection for flow cytometric and immunohistological analysis. Such systems are arguably the best immunological tools for detailed analysis of naïve T-cell activation *in vivo*.[24] The first study using this approach examined the activation of ovalbumin (OVA)-specific, DO11.10 transgenic CD4 T cells after subcutaneous injection of a *Salmonella* strain that was engineered to express OVA.[25] OVA-specific T cells were found to have proliferated in the local draining lymph node and acquired the ability to secrete interferon-γ (IFN-γ) by 5 days after *Salmonella*-OVA infection. This study therefore validated the usefulness of this methodology for examining the activation of *Salmonella*-specific CD4 T cells *in vivo*.

A similar approach, but using a more physiological route of infection, examined the activation of DO11.10 T cells in the Peyer's patch in response to oral *Salmonella*–OVA infection.[26] In this case, Peyer's patch OVA-specific T cells were found to expand and contract with slightly delayed kinetics compared to previous studies, perhaps reflecting a lower initial antigen load in lymphoid tissue when using the oral route. Immunohistology was used to physically locate OVA-expressing bacteria and activated OVA-specific CD4 T cells in the Peyer's patch. Interestingly, *Salmonella* remained in the subepithelial dome (SED) region of the tissue, while OVA-specific T cells were located exclusively in the interfollicular (IFR) T-cell area. These data imply that some form of antigen transport, from the SED to the IFR, is required to initiate T-cell activation in the Peyer's patch, as has been suggested in other model systems.[27]

Despite the utility and adaptability of using heterologous expression systems like *Salmonella*-OVA to track T-cell activation to microbial infection, there are also some limitations to this approach. The most obvious of these being that OVA is clearly not a natural microbial antigen. Thus, the data may not accurately reflect T-cell activation to a natural bacterial antigen. In order to characterize the response to a natural *Salmonella* epitope, a TCR transgenic adoptive transfer system for tracking CD4 T-cell responses to *Salmonella* flagellin was developed.[28] In agreement with earlier experiments using *Salmonella*–OVA,[26] *Salmonella* flagellin-specific T-cell activation was observed in the Peyer's patch after oral infection. Surprisingly, *Salmonella*-specific CD4 T cells were activated to express surface CD69 within 3 hr of oral infection and produced maximal levels of interleukin-2 (IL-2), 9–12 hr later.[28] The rapidity of this T-cell response suggests that the natural process of *Salmonella* antigen acquisition, processing and presentation can be accomplished within a couple of hrs after oral infection and has implications for the mechanism of antigen presentation to *Salmonella*-specific T cells in the Peyer's patch.

Previous work suggested that dendritic cells in the *Salmonella*-infected Peyer's patch may acquire bacterial antigens from infected macrophages that had been induced to

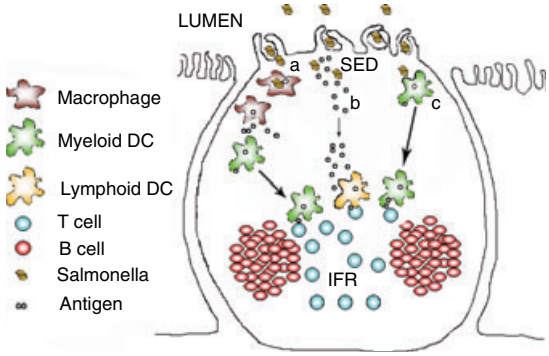

Figure 1. Possible pathways of antigen presentation in the Peyer's patch following *Salmonella* infection. (a) Myeloid dendritic cells (DC) lining the subepithelial dome (SED) acquire the *Salmonella* antigens released by apoptotic macrophages loaded with bacteria and migrate to the interfollicular region (IFR) to effectively present the antigen to T cells. (b) *Salmonella* entry into the Peyer's patch leads to the release of soluble bacterial antigens into the SED. These proteins quickly disseminate to the IFR in lymph fluid, are acquired by the resident lymphoid DC population, and subsequently presented to the T cells. (c) Myeloid DCs in the SED acquire *Salmonella* antigens directly following bacterial entry and migrate to the IFR for efficient antigen presentation.

undergo apoptosis (Fig. 1a).[29] However, it seems unlikely that the process of macrophage infection, apoptosis, engulfment by a myeloid dendritic cell in the SED, and subsequent T-cell activation could be accomplished within the short time frame noted in the flagellin–CD4 system. Therefore, it would be beneficial to consider some alternative models of *Salmonella* antigen acquisition and presentation in the Peyer's patch.

Perhaps the simplest model would be that soluble bacterial antigens are excreted into the SED lymph fluid, and that these flow rapidly to the IFR T-cell area without need for any cellular transport (Fig. 1b). These bacterial antigens could then be processed and presented by the resident lymphoid dendritic cell population in the IFR, already situated in close proximity to naïve T cells.[30] This model has the distinct advantage in that it proposes no anatomical scampering around the Peyer's patch by either dendritic or T-cell populations, and therefore has the potential to be more rapid than models involving any cellular chemotaxis. Furthermore, movement of lymph fluid from the SED to IFR would be in general agreement with currently understood models of the anatomy and lymph circulation within the Peyer's patch.[31] Adding a slightly unusual possibility to this simple model is the finding that protease digestion of *Salmonella* flagellin coincidentally gave rise to the exact minimal I-A^b binding epitope recognized by *Salmonella* flagellin-specific T cells[5,32] implying that this particular T-cell epitope is relatively protease resistant. Therefore, although processing by IFR resident dendritic cells seems more likely, is remains possible that *Salmonella* flagellin is transported to the IFR by lymph, processed by undefined extracellular proteases, to rapidly give rise to the required I-A^b binding epitope recognized by flagellin-specific T cells. Whatever the exact mechanism of peptide generation, the rapid movement of bacterial antigens to the IFR in lymph fluid seems a reasonable model that would explain the kinetics of *Salmonella*-specific CD4 T-cell activation noted *in vivo*.[28]

An alternative possibility is that myeloid dendritic cells within the SED engulf and process *Salmonella* antigens and subsequently carry them to T cells within the IFR (Fig. 1c). This is similar to model-A except that it requires no intermediary macrophage infection and apoptosis. Indeed we favour this particular model of antigen presentation, as CCR6-deficient mice that lack a myeloid dendritic population underlying the SED also display deficiencies in the activation of *Salmonella* flagellin-specific T cells in the Peyer's patch (Salazar-Gonzalez and McSorley, unpublished observation). Another study has already reported the relocation of SED dendritic cells to the IFR in response to oral *Salmonella* infection, although the rapidity of this process was not examined in any detail.[33] As flagellin itself has intrinsic proinflammatory properties, it may actually provide the trigger that initiates the exodus of dendritic cells from the SED.[5,34–37] In agreement with this hypothesis, flagellin stimulation of intestinal epithelial cells *in vitro* was found to influence the migration of dendritic cells through production of the chemokine CCL20.[38] To follow this idea further, it is worth considering the possibility that flagellin epitopes are preferentially processed and presented compared to other *Salmonella* proteins which lack proinflammatory properties.

It should be emphasized that each of these models of *Salmonella* antigen presentation in the Peyer's patch are not mutually exclusive, and the only natural *Salmonella* epitope that has been examined to date is found within a highly expressed, secreted antigen with unusual proinflammatory properties.[37,39] Future experiments will determine to what extent some combination of each of these models accounts for *Salmonella*-specific T-cell activation in the Peyer's Patch.

Expansion of *Salmonella*-specific effector T cells

Although *Salmonella*-specific CD4 T-cell activation is quickly initiated in mucosal lymphoid tissues, bacteria are able to escape this lymphoid tissue and penetrate to systemic sites, most notably the spleen, liver, and bone marrow.[40] In the face of intracellular microbial replication and dissemination, it is vitally important for the host that *Salmonella*-specific T cells are promptly expanded and acquire effector functions. A number of recent studies have examined the expansion of polyclonal T-cell responses following *Salmonella* infection. Experiments by

Mittrucker *et al*. noted the surprising finding that the majority of splenic CD4 and CD8 T cells in *Salmonella*-infected resistant mice display an activated phenotype.[41] Furthermore, many of these cells had gained the capacity to secrete IFN-γ in response to re-stimulation,[41] a known property of effector T cells.[42] In agreement with these data, other reports have described large numbers of *Salmonella*-specific CD4 and CD8 T cells following infection of susceptible mice with attenuated *Salmonella*.[43–45] Interestingly, these studies suggest that the peak frequency of *Salmonella*-specific CD4 T cells may be greater than 50% of all CD4 T cells.[44] Although such numbers are not unusual when assessing the CD8 response to viral infection[46,47] previous estimates of the clonal burst size of CD4 T cells have been much lower in other models.[48–50] Furthermore, one study has reported that CD4 T cells and CD8 T cells have intrinsic differences in their proliferative capacity.[51] However, the available data from *Salmonella* infection demonstrate that massive peak CD4 responses can be detected *in vivo*. Whether this large response actually represents extensive proliferation of *Salmonella*-specific naïve T cells, or the recruitment of an unusually large number of pre-existing pathogen-specific T cells, remains to be determined. It is possible that an elevated frequency of *Salmonella*-specific T cells will be found in the T-cell repertoire of uninfected mice due to cross-reactivity between *Salmonella* and endogenous gut flora antigens, although this has yet to be demonstrated experimentally.

Numerous studies have described the development of CD4 and CD8 T cells that secrete IFN-γ during *Salmonella* infection.[52,53] Indeed, one characteristic of the large frequency of *Salmonella*-specific CD4 T cells noted in recent studies is that many of these cells are able to produce IFN-γ *ex vivo*.[41,43–45] These data underline the importance of IFN-γ production to the generation of protective immunity in this model.[9] In addition to the development of effector cytokine production, many of these activated *Salmonella*-specific T cells acquire the capacity to migrate to non-lymphoid tissues such as the liver,[45] a major site of bacterial replication. Thus, during infection, a *Salmonella*-specific effector population expands rapidly in lymphoid tissues and redistributes effectively to the major non-lymphoid sites of bacterial infection. However, it should be noted that most of these studies have examined the effector function and migration of *Salmonella*-specific T cells in response to attenuated bacteria, and the T-cell response to virulent bacteria may differ. Indeed, a deficiency in the migration of *Salmonella*-specific T cells to non-lymphoid tissues was previously noted in mice infected with virulent *Salmonella*.[28] However, it is extremely difficult to examine T-cell effector function and migration when using a mouse model of acute infection that is rapidly fatal. Perhaps future studies using virulent *Salmonella* and antibiotic treatment will clarify whether T-cell effector function and migration is compromised following infection with virulent bacteria.

Given the reported magnitude of the polyclonal *Salmonella*-specific T-cell response[41,44,45] one might imagine that it would be easy to generate class-I and class-II tetramer reagents to track *Salmonella*-specific responses *in vivo*. However, as already noted above, the number of naturally defined class-I and class-II epitopes in the mouse model of *Salmonella* infection is somewhat limited. For CD4 T cells there are only three defined *Salmonella* epitopes, I-A^k/FliC 339-50,[54] I-A^b/FliC 427-41,[32] and I-A^d/SipC 381-94.[55] For CD8 T cells, two K^b peptide epitopes from outer membrane protein C[56] and an epitope presented by the class-Ib molecule Qa-1[57] have been described. Aside from the limited number of epitopes to choose from, the frequency of endogenous T-cell responses to each of these defined epitopes is either untested[54–57] or found to be surprisingly low.[32] Therefore, identification of new class-I and class-II *Salmonella* epitopes, or better characterization of existing epitopes, is required for the generation of tetramer reagents and a more complete analysis of the endogenous T-cell response to *Salmonella* infection.

It is not yet clear whether the large polyclonal population of *Salmonella*-specific T cells consist of a small number of highly expanded clones responding to a few major antigen specificities, or many small pools representing numerous different clonotypes. The antibody response in *Salmonella*-infected mice is clearly directed against many different target antigens, including flagellin, lipoproteins, and LPS.[58] It seems likely that the T-cell response will be similarly diverse. Flagellin remains the most consistently identified target antigen of *Salmonella*-specific CD4 T cells *in vivo*, and can confer limited protective immunity when used in a subunit vaccine formulation.[32,54,59,60] The most thoroughly studied CD8 T-cell response in the mouse model is directed against a GroEL peptide presented by the MHC class Ib molecule Qa-1.[11,57] CD8 T cells responding to GroEL display an interesting cross-reactivity to a peptide derived from mouse heat-shock protein-60, although the functional significance of this cross-reactivity for *Salmonella* infection is unclear. Recent innovative work has managed to identify five new *Salmonella* proteins that are controlled by highly expressed, *in vivo* inducible promoters.[61] The rationale for this approach was that by identifying *Salmonella* proteins with these attributes, the most likely targets for recognition by the adaptive immune response would also be discovered. Indeed, this study demonstrated that two of these proteins, Mig-14 and SseB can mediate protective immunity when used as a subunit vaccine. It therefore seems likely that T cells responding to these antigens will comprise a fraction of the large *Salmonella*-specific CD4 cell response described above.[41,44,45] However, at present the *Salmonella* target antigens recognized by T cells remain incompletely defined.

The role of B cells in relation to the expansion of *Salmonella*-specific T cells remains unclear. Aside from the requirement for B cells in mediating protective immunity, it has also been reported that B-cell antigen presentation to T cells can augment the *Salmonella*-specific CD4 response.[12,62] During primary *Salmonella* infection, *Salmonella*-specific T cells initially expand within the T-cell area of lymphoid tissues and are only observed to penetrate B-cell follicles at a later stage.[23,28] Therefore, anatomically, B-cell antigen presentation to T cells is likely be a secondary event to the initial activation mediated by dendritic cells in the T-cell area of lymphoid tissues. Furthermore, although vaccinated B-cell deficient mice succumb to infection with virulent *Salmonella*, they also display a reasonable degree of immunity when re-exposed to attenuated *Salmonella*.[13] The most likely explanation for this protective immunity is that a *Salmonella*-specific effector T-cell response can be generated in the absence of B cells, although it is possible that it may be somewhat compromised when compared to wild-type mice. It would also seem likely that B-cell presentation to T cells would be most clearly observed during a secondary infection, where *Salmonella*-specific B cells are at an elevated frequency, or following high-dose *Salmonella* challenge, when bacteria have been found to associate more readily with B cells.[43] However, at present these issues remain incompletely resolved experimentally and it is possible that B cells contribute to antigen presentation during *Salmonella* infection.

Salmonella-specific memory T cells

Long-term immunity to pathogens is believed to reside within the memory pool of T and B lymphocytes that can persist for the lifetime of an individual.[63] Despite the current availability of live attenuated, and polysaccaride vaccines, typhoid fever remains a significant health care problem in developing nations. Therefore, a greater understanding of the generation of immunological memory during *Salmonella* infection is critical to improving current vaccine strategies.

Immunization of both humans and mice with live attenuated *Salmonella* vaccine strains, leads to the generation of long lasting immunity and resistance to re-challenge.[15,64] The ability of expanded polyclonal CD4 and CD8 T cells to secrete IFN-γ in response to *Salmonella* antigens persists in the mouse model for at least 6 months after vaccination (Srinivasan and McSorley, unpublished observation). Therefore, the generation of immunological memory and protective immunity can be a robust phenomenon after exposure to attenuated *Salmonella*.

The *Salmonella* flagellin-specific TCR transgenic adoptive transfer system has been used to examine the development of memory CD4 T cells in *Salmonella*-infected mice.

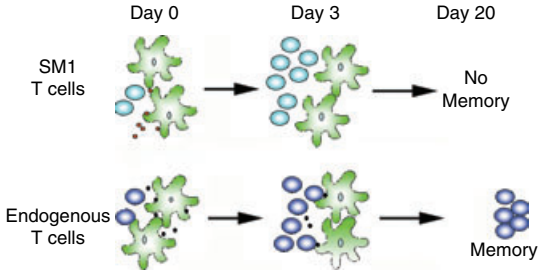

Figure 2. A model to explain the survival of certain antigen-specific *Salmonella*-specific memory cells. SM1 cells are initially activated (Day 0), expand (Day 3), but fail to get selected into the memory pool because of an inadequate supply of flagellin peptide as the infection progresses *in vivo*. Endogenous *Salmonella* specific T cells directed against other antigenic epitopes expand and are successfully selected to persist because of maintained antigen presentation.

In other model systems the adoptive transfer of TCR transgenic T cells inhibits the expansion of endogenous T cells with identical peptide/MHC specificity.[65] However, the expansion of a large endogenous CD4 response to *Salmonella* actually inhibited the persistence of the SM1 T cells *in vivo*.[44] The basis of this competition is not yet clear, and may involve competition for T-cell growth or survival factors such as IL-7.[66,67] However, we favour a modification of the model of Rollenhagen *et al.*[61] where persistent antigen presentation of *Salmonella* epitopes *in vivo* may actually select clonotypes for survival to the memory pool (Fig. 2). It has been reported that flagellin expression is rapidly down-regulated by *Salmonella* growing in macrophages.[68] Therefore, the presentation of this particular *Salmonella* epitope may be limiting as the infection progresses *in vivo*. In the face of intense competition with a massive endogenous *Salmonella*-specific pool directed against other epitopes that are highly expressed *in vivo*, SM1 T cells may fail to be selected for survival to the memory pool. Experiments are currently underway in our laboratory to test this hypothesis. It should be noted, however, that such a model contrasts with previous work with pathogen-specific CD8 T cells, where the peak expansion frequency of a given T-cell clonotype correlates well with the memory frequency.[46]

These limited observations on memory T-cell development have some implications for vaccination against typhoid fever. For example, the current dogma that live attenuated *Salmonella* vaccines will always provide better protective immunity than a subunit vaccine may be incorrect. The success of live attenuated vaccines may have more to do with the selective pressure placed upon the expanded *Salmonella*-specific T-cell pool, than with the nature of the vaccine itself. In other words, if the antigenic targets of the S*almonella*-specific memory T-cell response could be elucidated, immunization with these individual proteins may have the potential to be as protective as a live attenuated strain of *Salmonella*. Indeed,

the recent studies by Rollenhagen *et al*. appear to validate this hypothesis.[61] This point is not trivial from a vaccine standpoint, since the live attenuated typhoid vaccine is not currently licensed in the US for children under the age of 6 years old, primarily because of safety concerns associated with a live vaccine.[69,70] Therefore, the generation of a subunit vaccine that could reproduce the efficacy and long-term protection of a live *Salmonella* vaccine would represent a considerable advance in vaccine development, as it would be possible to target the most vulnerable demographic with prophylactic intervention.

Evasion of *Salmonella*-specific T cell responses

Intracellular pathogens have developed numerous strategies to gain access to the host and to survive under the constant glare of a hostile immune system. *Salmonella* have been variously described to actively interfere with antigen processing and presentation[71–74] induce apoptosis of antigen-presenting cells[29,75–79] and to generate an immunosuppressive environment *in vivo*.[80–82]

The fact that *Salmonella* can infect dendritic cells of diverse origin[43,79,83–86] may indicate that the bacteria have the capacity to directly inhibit the priming of naïve T cells. Indeed, one elegant study recently identified the *Salmonella yej* operon as encoding a bacterial transporter system that interferes with MHC class-I antigen presentation in macrophages.[73] Although the exact mechanism of this interference is not understood, it was proposed that this transporter system might prevent peptide loading of phagosomal MHC class I molecules by flooding the vacuole with competing short peptides. Another recent report demonstrated that *Salmonella* can avoid lysosomal degradation and impair the antigen presentation properties of dendritic cells.[74] Interestingly, this process was blocked in the presence of *Salmonella*-specific immunoglobulin G, which binds FcgR on dendritic cells, and effectively targets bacteria to lysosomes. These data may shed light on the requirement for serum antibody in protective immunity to *Salmonella* infection. The role of antibody may actually have less to do with the opsonization and clearance of extracellular bacteria[87] and more to do with the inhibition of a specific bacterial process that can interfere with naïve T-cell activation.

The notion that *Salmonella* may interfere with the activation of naïve T cells fits nicely with data using the *Salmonella* flagellin-specific adoptive transfer system. Although *Salmonella*-specific T cells are efficiently activated following infection, this activation is highly dose dependent.[88] Indeed, flagellin specific SM1 cells were found to be totally unresponsive after low dose infection with virulent *Salmonella*, despite extensive bacterial replication *in vivo*.[88] Infection with a live vaccine strain of *Salmonella* also demonstrates the same sensitivity to challenge dose (Srinivasan and McSorley, unpublished data). These data are reminiscent of human vaccine trials with attenuated *Salmonella* that are usually poorly immunogenic unless administered in multiple doses with large numbers of bacteria.[64,89] It is possible that natural exposure to low numbers of bacteria avoid detection by the adaptive immune system, and that this can be attributed to some of the evasion mechanisms discussed above. If this turns out to be the case, the evasion properties of *Salmonella* vaccine strains clearly need to be elucidated and inhibited, as has already been described in the case of the *yej* operon.[73]

Conclusion

Salmonella induce rapid and robust T-cell activation following infection of the mammalian host. The exact processes involved in the initial activation, expansion, and memory development of *Salmonella*-specific T cells are beginning to be unravelled. It seems likely that the speed and magnitude of the *Salmonella*-specific T cell effector response has been underestimated and that this particular model may be particularly suited to understanding T-cell activation in response to infection. However, clearer identification of target antigens and an understanding of bacterial processes to inhibit T-cell activation are required in the future. Addressing these issues is likely to lead to significant improvements in current typhoid vaccine formulations, or the generation of novel typhoid vaccines that will be safer and more immunogenic than those currently available.

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