Th1 dominance in the immune response to live Salmonella typhimurium requires bacterial invasiveness but not persistence

Achal Pashine, Beena John, Satyajit Rath, Anna George and Vineeta Bal
National Institute of Immunology, Aruna Asaf Ali Road, New Delhi 110 067, India

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Abstract
Factors responsible for the predictable generation of Th1 or Th2 immune responses to microorganisms in vivo are not well characterized, although the ability of antigen presenting cells (APC) to provide co-stimulation, the kinetics of MHC–peptide ligand generation as well as the cytokine environment are all considered important factors for the differential Th1/Th2 priming of T cells. Our earlier findings of an IFN-γ-dominant, Th1-type response to live Salmonella typhimurium (Stm) and a Th2-type response to killed Stm suggested that persistence of viable bacteria might be an important factor in the generation of IFN-γ-dominant responses. Using genetically susceptible and resistant strains of mice to limit bacterial replication and persistence in vivo, we show that mice of the Ity$r$ genotype, capable of a 10-fold better clearance of Stm, mount an IFN-γ-dominant immune response following immunization with live Stm similar to that in the Ity$s$ strain. Further, metabolically defective mutants of Stm, aroA and purA, when used in the live form, also elicit IFN-γ-dominant immune responses similar to the wild-type Stm strain despite their inability to proliferate in vivo. While a laboratory strain of Escherichia coli, which is antigenically cross-reactive but non-invasive, elicits hardly any IFN-γ in immune responses, an invasive strain of E. coli induces an IFN-γ-dominant response. These data together indicate that, while entry of bacteria into macrophages is likely to be critical for the generation of IFN-γ-dominant immune responses, their persistence is not.

Introduction
CD4 T lymphocytes secrete a variety of cytokines on activation. Depending on the cytokines they secrete they are classified into Th1 or Th2 cells (1,2). Development of such responses against microbial antigens has been a subject of great interest, especially because the rules predicted for such responses based on T cell clonal data do not necessarily apply to the situation in vivo (3–5). In the past few years there have been extensive reports about possible factors responsible for the Th1/Th2 dichotomy in T cell responses following exposure to parasite antigens in vivo (6–9). The ability of professional antigen-presenting cells (APC) to provide co-stimulation during priming has been considered one of the important host factors responsible for differential priming of T cells (10,11). The kinetics of expression of CD80 (B7-1) and CD86 (B7-2) molecules on the APC, for example, have been shown to affect the Th1/Th2 balance significantly (12). In addition to the extensive documentation of the role of IL-4, IL-5 and IFN-γ (1,10), it has also been shown that the presence of IL-12 early during the course of infection and/or immunization also affects the Th1/Th2 balance in favor of Th1 responses (13,14). Apart from the role of cytokines and co-stimulatory factors, the affinity of a peptide for the presenting MHC molecule has been shown to affect the Th1/Th2 balance (15,16) suggesting that peptide–MHC ligand levels, and therefore pathogen dose might be a significant factor in the control of cytokine commitment of T cells. Work on leishmanial infections has shown that certain mouse strains are more prone to Th1- or Th2-like responses (3). In addition, certain parasitic antigens have been reported to be dominantly contributing to the development of Th1- or Th2-like responses as well. In schistosomal infections, antigens from two different stages of the life cycle of the parasite have been reported to generate the differing cytokine responses (17). Brugia malayi infection in mice also results in stage-dependent immune
responses (18). Thus, a variety of factors have been shown to contribute to the development of T\textsubscript{h}1-like immune responses and the basis for the differential commitment has been argued to be different in various cases.

We have reported earlier that immunization with live *Salmonella typhimurium* (Stm) given i.p. or orally brings about an IFN-\(\gamma\)-dominant immune response in BALB/c mice, while killed Stm generate a T\textsubscript{h}2-like response (19, 20). Stm is a facultative intracellular bacterial parasite that prevents phagolysosomal fusion and multiplies within macrophages (21). As a result, live Stm survive inside phagocytic cells and are detectable in lymphoid tissues for long periods of time. Based on our earlier findings of immunization with live Stm resulting in IFN-\(\gamma\)-dominant immune responses (19), it could be argued that such persistence of live Stm *in vivo* could contribute to the T\textsubscript{h}1-like outcome of immunization. In order to address this issue, we have used a variety of strategies such as genetic susceptibility or resistance of various mouse strains to *Salmonella* and strains of Stm which are deficient in their ability to multiply in the host. Using these, we have analyzed the effects of immunization with live or heat-killed Stm in an effort to examine the effects of persistence of replicating antigen on effector immune responses.

**Methods**

**Mice**

Male or female BALB/c (H-2\textsuperscript{d}, *Ity\textsuperscript{a}*), C57BL/6 (H-2\textsuperscript{b}, *Ity\textsuperscript{b}*), and C3H.SW (H-2\textsuperscript{b}, *Ity\textsuperscript{b}*), and *Salmonella* C.D2 (H-2\textsuperscript{d}, *Ity\textsuperscript{a}*), which are BALB/c congenic for the *Ity* locus, a kind gift from Professor E. Skamene (McGill University, Montreal, Canada), were also similarly bred. All mice were used at 6–10 weeks of age. Six to 10 mice were used in each group. Bleeding was done from retro-orbital venous plexus under inhalation anaesthesia, and separated sera were used for antibody titer measurements. Most assays were carried out between 14 and 50 days after immunization, and our earlier data show that qualitatively the results remain stable over this period (19).

**Bacteria**

A clinical isolate of *S. typhimurium*, Stm-754 (19), was used as the wild-type strain for this work. The LD\textsubscript{50} of Stm-754 for BALB/c and C57BL/6 mice was 1.4–2.0\(\times\)10\textsuperscript{4} bacteria given i.p. (19, 22). Two auxotrophic mutants of Stm were used, SL3235 (aroA), which is a kind gift from Dr K. Sanderson (*Salmonella* Genetic Stock Center, Calgary, Canada) and BRD150 (purA), which is a kind gift of Dr G. Dougan (Imperial College of Science Technology and Medicine, London, UK). A laboratory strain of non-invasive *E. coli* was a kind gift of Dr A. Y. Rudensky (University of Washington, Seattle, WA). Enteroinvasive *E. coli* (ATCC 43892) was purchased from ATCC (Rockville, MD). Bacterial stocks were maintained in glycerol broth at –70°C. A clinical isolate of *S. typhimurium*, Stm-754 (19), was used as the wild-type strain for this work. The LD\textsubscript{50} of Stm-754 for BALB/c mice, while killed Stm generate a T\textsubscript{h}2-like response (19, 20).

In a boiling waterbath for 45 min and efficiency of killing was confirmed by the absence of bacterial growth in plated suspension. Killed bacteria were suspended in PBS and sonicated in the presence of 10 mM PMSF (Boehringer, Mannheim, Germany) and bacterial protein extracts prepared by sonication as described (19). This is referred to as bacterial sonicate.

**Estimation of c.f.u.**

Spleens were teased apart in 0.1% sodium taurocholate (Hi-Media). Appropriate dilutions were plated either on Salmonella-Shigella agar (Hi-Media) or LB agar plates. Colonies were counted after incubation at 37°C for 24 h and c.f.u. per spleen were determined.

**Immunization**

Mice were immunized i.p. A single dose of live or heat-killed bacteria was used for immunization as described for each experiment.

**Delayed-type hypersensitivity (DTH) response**

Mice were given 50 \(\mu\)l PBS containing 50 \(\mu\)g bacterial sonicate in one hind footpad and 50 \(\mu\)l PBS in another hind footpad as a control. Footpad thickness was measured using a spring loaded caliper (Mitutoyo, Tokyo, Japan) and expressed as increase over the control reading in mm as mean \(\pm\) SE.

**IgG isotype analysis by enzyme immunoassay (EIA)**

Polyvinyl chloride plates (Nunc, Roskilde, Denmark) were coated with 10 \(\mu\)g/ml of Stm or *E. coli* sonicate. Sera from immune mice were titrated followed by polyclonal goat anti-IgG1 and anti-IgG2a (Sigma, St Louis, MO) antibodies at the optimal dilutions. The bound antibodies were detected by rabbit anti-goat IgG-peroxidase with o-phenylenediamine (Sigma) and hydrogen peroxide (Merck, Mumbai, India) as revealing agents. Absorbances were read at 490 nm in a microplate reader (EL340, Biotek, Winooski, VT). Log serum dilutions required to reach half-maximal absorbance were estimated and used to calculate ratios of IgG1/IgG2a.

**T cell proliferation assay**

Mice were sacrificed 8–10 days after immunization, splenectomies were taken out aseptically in DMEM (Life Technologies, Gaithersburg, MD) containing L-glutamine (Life Technologies), antibiotics (Hi-Media) and 10% FBS (Life Technologies), and single-cell suspensions were prepared. Cells were added at 3–5\(\times\)10\textsuperscript{5} cells/well with varying doses of antigen as indicated in triplicate cultures in 96-well plates in 200 \(\mu\)l final volumes and incubated at 37°C in a humified incubator with 7.5% CO\textsubscript{2} for 60 h. At this time 100 \(\mu\)l of culture supernatant was removed and frozen, the wells were replenished with fresh medium, and incubation continued. Plates were pulsed with 0.5 \(\mu\)Ci [\(^3\)H]thymidine (NEN, Boston, MA) for the last 12–16 h of an 84–96 h assay, harvested on glass fiber filtermat (Wallac, Turku, Finland) and analyzed in a scintillation counter (Betaplate; Wallac). Results are expressed as mean \(\pm\) SE of triplicate cultures.

**Cytokine assays**

The culture supernatants were freeze-thawed before being subjected to EIA for IFN-\(\gamma\) and IL-10. Manufacturers’ instruc-
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In Ity-disparate H-2d strains, Ity⁺ (C.D2) mice clear Stm more rapidly than Ity⁻ (BALB/c) mice. Splenic c.f.u. (mean ± SE) are shown for groups of three mice sacrificed at each time point after i.p. injection of ~1×10⁵ live Stm-754. The data are representative of three experiments.

**Results**

**Clearance of live Stm in Ity⁺ and Ity⁻ strains**

It has been reported that NK cells and γδ T cells in the Ity⁺ strain release IFN-γ early during the course of infection (23,24), and hence macrophages from Ity⁺ strains clear bacteria faster than those from the Ity⁻ strain. In order to examine the role of differential bacterial killing at early time points on the outcome of immunization with Stm, experiments were carried out in two pairs of mouse strains differing at the Ity locus. C3H.SW and C57BL/6 share MHC haplotypes (H-2b) but are disparate for the Ity locus. C.D2 and BALB/c mice (H-2d) are also congeneric at the Ity locus.

All strains were given ~1×10⁵ live Stm-754 i.p. Mice were sacrificed at 24, 48 and 60 h after injection, and the number of viable bacteria measured in the spleen as c.f.u. Figure 1 shows a representative experiment in the H-2d strains where the load of bacteria per spleen is at least 10-fold lower in Ity⁺ mice, confirming that bacteria are cleared more efficiently in the Ity⁺ strains used (25). Ity⁺ mice showed a consistent 10- to 30-fold better clearance in all the experiments.

**Analysis of the Tₘ₁/Tₘ₂ balance in Ity⁺ and Ity⁻ strains**

In order to assess the effect of the 10-fold difference in bacterial load attained in vivo following infection of Ity⁺ and Ity⁻ mice on the development of effector T cell responses, we immunized various strains of mice with ~1×10⁵ live Stm-754 or ~1×10⁸ heat-killed Stm-754 i.p., as used earlier (19).

Figure 2(A and B) shows that both in the H-2b and H-2d pair of strains, mice immunized with live Stm mount significantly higher DTH on day 28 of immunization as compared to mice immunized with killed Stm and the pattern is independent of Ity allele. Mice were bled on day 21 of immunization, and the relative levels of anti-Stm antibodies of IgG1 and IgG2a isotypes were compared between the groups as indirect indicators of IL-4 and IFN-γ dominance respectively. Figure 2(C and D) shows the relative proportions of these antibodies in the form of ratios. In all strains of mice, immunization with live Stm resulted in lower IgG1/IgG2a ratios as compared to immunization with killed Stm.

Splenocytes from variously immunized mice were stimulated in vitro with Stm sonicate, and culture supernatants were collected and analyzed for their cytokine content by EIA. Since IL-4 was below the detection limit using commercially available EIA, IL-10 from antigen-stimulated cultures was used to indicate T cell priming to the given antigenic mixture. While non-T cells are also known to secrete IL-10, the fact that no IL-10 was detected in these cultures in the absence of antigen, nor was any IL-10 detected in antigen-stimulated cultures of splenic cells from non-immunized mice, established that the IL-10 being detected in these experiments was
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Fig. 3. In both Ilyr (squares) and Ilys (circles) mice, live Stm immunization (hollow symbols) results in greater dominance of IFN-γ as compared to heat-killed Stm immunization (filled symbols). Ilyr and Ilys mice from H-2b (A and B) and H-2d (C and D) strains show higher levels of IFN-γ secretion from splenocytes stimulated with Stm sonicate in vitro when immunized with live Stm as compared to killed Stm (A and C). (B and D) Secretion of IL-10 from the same culture supernatants. Background secretion of IFN-γ and IL-10 in the absence of antigen (T cells + APC) was below the detection limit of the assay. The data are representative of three independent experiments.

of immune T cell origin. IFN-γ served as an indicator of Th1 dominance or otherwise of the immune response. As shown in Fig. 3(A and B), heat-killed Stm immunization generates relatively far less IFN-γ as compared to immunization with live Stm and this is true in all four mouse strains tested, regardless of their Ily status. These data clearly indicate that the reduction in numbers of live Stm surviving in Ilyr strains does not affect the qualitative outcome of the immune response in either MHC haplotype tested.

Analysis of the Th1/Th2 balance generated by metabolically deficient Stm mutants

Ity-disparate strains provided a system where resistant strains have a 10-fold lower load of live Stm than sensitive strains within the first week of immunization. We also used two auxotrophic mutant strains of Stm to study the outcome of an even larger decrease in the effective bacterial load on the resultant Th1 dominance. The aroA mutant of Stm lacks an essential enzyme necessary for the synthesis of aromatic amino acids and hence does not replicate in vivo, while the purA mutant has a defect in its purine biosynthetic pathway leading to similar auxotrophy (26). Spleens of mice immunized with live Stm-aroA or Stm-purA (~10^5) given i.p. show no viable bacteria at the end of 48 h (data not shown). Thus, there are no detectable replication competent bacteria in the body by 48 h in contrast to the results in Fig. 1 with Stm-754. BALB/c mice were immunized i.p. with either live or heat-killed Stm-754, Stm-aroA or Stm-purA as described above. Figure 4(A) shows that a good DTH response is detectable on day 36 in mice immunized with live Stm-aroA or Stm-aroA but not in those given killed Stm-aroA or Stm-purA. Figure 4(B) shows the relative ratios of IgG1/IgG2a in sera from Stm-754 and Stm-aroA immune mice. It can be seen
that regardless of the strain of Stm used, mice immunized with live bacteria show lower IgG1/IgG2a ratios than their counterparts immunized with killed bacteria. The titrations for IFN-γ and IL-10 in the Stm sonicate-activated culture supernatants of splenocytes from these mice shown in Fig. 5(A and B respectively) demonstrate that for every strain of Stm used, live bacterial immunization triggers higher levels of IFN-γ secretion from immune T cells than heat-killed bacterial immunization does. Thus, in spite of very rapid clearance of live auxotrophic Stm in vivo, the mice mount a Th1-like immune response to them, suggesting that persistence of live Stm beyond the first 48 h may not be required for effective priming of T cells to the Th1 phenotype. Immune response differences between the immune responses to the various Stm strains were not consistently observed.

Immune responses using equal numbers of live or killed Stm

Because of the virulence of the Stm-754 strain and of its persistence in vivo, it has not been possible to administer effectively identical numbers of live and heat-killed Stm-754 for immunization. Therefore it could be argued both that ~1×10^8 heat-killed bacteria constituted a very high antigenic load as compared to ~1×10^3 live bacteria, and that live Stm-754 could multiply and persist in vivo for long periods of time and hence a reliable estimate of antigenic load could not be obtained. To overcome this problem, we used identical doses of live and heat-killed Stm-aroA (~1×10^8) for immunization. Figure 6(A) shows that the IgG1/IgG2a ratio of Stm-aroA-specific antibodies is higher in mice immunized with heat-killed Stm-aroA than in live Stm-aroA immune mice. Figure 6(B and C) shows the cytokine profile of splenic cells from these animals when stimulated in vitro with Stm sonicate. IL-10 is produced by T cells from mice immunized with both live and killed Stm-aroA; however, IFN-γ is close to background level in culture supernatents from killed Stm-aroA immune mice, even at antigen doses where the IL-10 produced is equivalent to that produced at an IFN-γ-generating dose by live Stm-aroA-immune cells.

Cytokine profiles in immune responses to live E. coli

The absence of any need for persistence of live bacteria beyond 48 h for triggering a Th1 response raised the possibility that immunization with any Stm-related live bacterial species can result in a similar IFN-γ-dominant immune response. To test this, we used a laboratory strain of E. coli for comparison. Stm and E. coli belong to the same family, i.e. Enterobacteriaceae, and have a very high degree of genomic sequence homology (27). However, Stm is a known facultative intracellular parasite while E. coli is not. BALB/c mice were immunized i.p. with ~1×10^8 live or ~1×10^8 killed E. coli or Stm-754. As shown in Fig. 7, mice immunized with live Stm-754 show a strong DTH on day 21 but mice immunized with live E. coli, heat-killed E. coli or heat-killed Stm show negligible DTH.

Anti-E. coli-immune responses in E. coli-immunized mice were confirmed by proliferative responses detected upon stimulation of splenic T cells in vitro with E. coli or Stm sonicate (data not shown). The cytokine responses of immune cells from these groups to both homologous and heterologous bacterial sonicates are shown in Fig. 8. T cells from mice immunized with live E. coli do not secrete detectable levels of IFN-γ (Fig. 8A), though they do secrete high levels of IL-10 (Fig. 8B), regardless of whether E. coli or Stm sonicate is used for re-stimulation in vitro. On the other hand, live Stm-754-immune mice show high IFN-γ-secretory capacity whether Stm or E. coli sonicate is used for recall (Fig. 8A and B). Cytokine responses of the mice immunized with killed E. coli or Stm are shown in Fig. 8(C and D), showing indistinguishable Th1/Th2 balances regardless of bacteria used for immunization or recall. Thus, the Th1/Th2 balance generated even against antigens cross-reactive between E. coli and Stm varies depending on whether live E. coli or Stm is the immunogen.

However, Stm and E. coli are separate species. We therefore also tested a known invasive strain of E. coli to immunize mice and compared the immune responses generated with those induced by the non-invasive laboratory strain of E. coli used. Figure 9(A) shows levels of IFN-γ produced by immune splenocytes from BALB/c mice in response to live and heat-
Fig. 7. Immunization with live non-invasive E. coli does not produce a Th1-dominant immune response. (A) DTH responses in BALB/c mice immunized with live or heat-killed Stm (hatched bars) and non-invasive E. coli (hollow bars) in response to homologous bacterial sonicate. The results are representative of three independent experiments.

Discussion
The ability to mount differential T cell responses making either IFN-γ or the IL-4/5/10 group of cytokines dominantly is crucial for optimization of protective immunity against infectious agents. However, the predictable development of Th1- or Th2-like immune responses in vivo to microbial organisms is still elusive. We have previously shown that live Stm trigger a Th1-dominant response as compared to killed Stm (19). Facultative intracellular parasites like Mycobacteria, Leishmania, Listeria and Salmonella enter phagocytic cells and survive intracellularly using various strategies to evade their destruction. Virulent mycobacterial strains, but not avirulent ones, are taken up by mannose receptors on macrophages (28). Salmonellae also promote their uptake in macrophages by macropinocytosis (29). Once inside the macrophages, Stm and mycobacteria evade phagolysosomal fusion within macrophages (21,30), thereby permitting intracellular persistence. Live Stm also delay and reduce phagosome acidification, thus helping their intracellular survival (31). The ability to persist intracellularly is considered to be one of the factors contributing to the virulence of the parasites. E. coli expressing certain genes from mycobacteria have been shown to invade macrophages and survive in the...
intracellular environment unlike the parental E. coli strain (32). In case of listerial infections, the presence of listeriolysin is important for promoting bacterial invasion into the cytosol (33). Facultative intracellular parasites thus use various strategies for their survival inside the host phagocytic cells. Therefore, it was pertinent to ask if persistence of live bacteria in phagocytes was essential for the generation of T1-like immune responses.

In our first approach, we have used mouse strains which have the same MHC haplotype but differ at the Bcg/ity/Lsh loci. Nrpm1 is documented to be the major candidate gene for these loci (34). Because of the presence of Nrpm1, tissue macrophages from ity' strains of mice restrict the proliferation of parasites during the early stage of infection as compared to those from ity'' mice (35). This is probably achieved by controlling the transport and/or metabolism of oxidized nitrogen radicals which are crucial for the microbicidal activity of macrophages (35). In addition, ity' macrophages are reported to have higher levels of activation markers as compared to ity'' mice possibly due to higher levels of IFN-γ found early on during the course of infection in ity'' mice. The two factors together may be contributing to the rapid clearance of bacteria. The bacterial load in spleens of ity' mice is 10-fold less very early after injection than in ity'' mice (Fig. 1). This decrease in bacterial load during the early stages of T cell priming does not modify the cytokine profile of the effector T cell response (Figs 2 and 3), as would be expected from earlier reports (25,37). In another approach, we have used metabolically deficient strains of Stm for immunization. This reduces the in vivo load of live bacteria even further and there are no viable bacteria recoverable from spleen after 48 h. Even such a drastic decrease in the persistence of live bacteria does not affect the qualitative outcome of the immune response (Figs 4 and 5). Thus, there appears to be no requirement for Stm persistence for generating IFN-γ-dominated immune responses.

Unlike Stm, E. coli does not behave as a facultative intracellular parasite, although Stm and E. coli belong to the same family, Enterobacteriaceae. However, certain genes can endow E. coli with enteroinvasive properties (38-40). Such invasive strains of E. coli are reported to invade a variety of cell types (39,40). Our results (Fig. 8) show that immunization with live, non-invasive E. coli triggers T cells capable of secreting high levels of IL-10 but IFN-γ secretion is negligible, while live Stm-immune cells secrete large quantities of IFN-γ. In contrast, immunization with live, invasive E. coli can trigger IFN-γ-secreting T cells (Fig. 9). These data indicate that the ability of live Stm or live invasive E. coli to enter APC may be crucial for triggering a T1-like immune response.

Stm and E. coli share many antigens and show extensive genomic sequence homology (27). Interestingly, T cells from mice immunized with live, non-invasive E. coli, when challenged in vitro with Stm sonicate, still do not secrete IFN-γ but secrete IL-10, while T cells from live Stm-immune mice respond even to E. coli sonicate by dominant IFN-γ secretion (Fig. 8). Thus, the T1/T2 balance even against cross-reactive epitopes is determined by the invasiveness of the immunizing bacteria. The ability of live Stm and invasive E. coli to reach the APC, survive intracellularly and/or modify the intracellular environment could be contributing to the priming of IFN-γ-secreting T cells.

Salmonella are known to enter phagocytic cells through receptor-mediated uptake (41). It has been shown that the intracellular pathways followed after phagocytosis, receptor-mediated endocytosis and macroinocytosis can be different (30,42). Whether such differing intracellular pathways followed by the ingested material can result in differential antigen processing and/or activation of APC is not yet clear and these may be potential factors for the altered T1/T2 balance seen. After rapid macroinocytosis, Stm can persist in the cytoplasm for a much longer period than ordinary phagosomes, thus, helping longer survival of Stm inside the cell. Phago-lysosomal fusion followed by a decrease in the pH of the vacuole is also delayed by live Stm as compared to killed Stm (31). Thus, differences in the handling of live and killed Stm intracellularly might be responsible for differences in the efficiency of antigen processing inside the APC, although our data also suggest that prolonged intracellular survival of the bacteria is not needed to trigger a T1 response.

The density of peptide–MHC complexes on APC has been argued to affect T1/T2 balance (15,43). To examine the role played by ligand density in the present system, we used identical numbers of live and killed Stm-aroA for immunization. Since Stm-aroA do not multiply in vivo, the bacterial load can be argued to be reasonably comparable. Figure 6 shows that such a dose equivalence still causes differential T1/T2 commitment. Comparable antigenic load can, theoretically, result in comparable peptide–MHC complex densities, which in itself may not be a crucial factor contributing to the T1/T2 dichotomy of immune response in this case. However, other data from our laboratory using pure protein antigens suggest that the kinetics of appearance and persistence of peptide–MHC complexes on APC, rather than density, alone may influence the T1/T2 balance (44). Whether differential handling of live and killed Stm in phagocytes can cause such differences in the kinetics of appearance and persistence of peptide–MHC complexes is not yet clear. The T1/T2 balance in vivo has been argued to be controlled not only by the density of peptide–MHC complexes (15,43), but also by the co-stimulatory signals provided by APC to T cells (12,13), and by cytokines like IL-4, IFN-γ and IL-12 (10,11). Accessory co-stimulatory signals have been shown to be altered on macrophages by live Stm (45) and IL-12 is also induced (46,47) although bacterial lipopolysaccharide, which is a major inducer of IL-12 from APC, would be equivalent in equal doses of live and killed Stm.

Together, our data suggest that cellular invasion is necessary during immune priming for effective generation of T1-like immune responses, but that persistence of live bacteria beyond 48 h is not required. Thus, whether it is peptide–MHC ligand densities or co-stimulatory alterations that permit live Stm to trigger a T1 response, these factors are likely to be determined either during or very soon after entry into APC.

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Abbreviations

APC antigen-presenting cells
DTH delayed-type hypersensitivity
EIA enzyme immunoassays
LB Luria–Bertani
Stm Salmonella typhimurium

References


