Cellular requirements for systemic control of *Salmonella Typhimurium* infections in mice

Running title: Role of lymphocytes in immunity to *Salmonella enterica*

Andreas Kupz¹, ², ³, Sammy Bedoui¹ and Richard A. Strugnell¹, #

¹Department of Microbiology and Immunology, The University of Melbourne, Parkville, 3010 VIC, Australia

²Max Planck Institute for Infection Biology, Charitéplatz 1, 10117, Berlin, Germany

³Queensland Tropical Health Alliance Research Laboratory, James Cook University, Cairns Campus, McGregor Road, Smithfield QLD 4878, Australia

Address correspondence to:

Dr. Andreas Kupz, Max Plank Institute for Infection Biology, Charitéplatz 1, 10117, Berlin, Germany. Phone: +49 30 28460 520, Fax: +49 30 28460 505, kupz@mpiib-berlin.mpg.de

Prof. Richard Strugnell, Department of Microbiology and Immunology, The University of Melbourne, Gate 11, Royal Parade, Parkville, 3010, Victoria, Australia. Phone: +61 3 8344 9916, Fax: +61 3 9347 1540, rastru@unimelb.edu.au
Abstract

The rational design of vaccines requires an understanding of the contributions of individual immune cell subsets to immunity. With this understanding, targeted vaccine delivery approaches and adjuvants can be developed to maximize vaccine efficiency and to minimize side effects (S.H.E. Kaufmann, M.F. Good and D.L. Doolan, Immunity 33:555-577, 2010; T. Ben-Yedidia and R. Arnon, Human Vaccines 1:95-101, 2005). We have addressed the contributions of different immune cell subsets and their ability to contribute to the control and clearance of the facultative intracellular pathogen *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) in a murine model. Using a systematic and reproducible model of experimental attenuated *S. Typhimurium* infection, we show that distinct lymphocyte deficiencies lead to one of four different infection outcomes – clearance, chronic infection, early death or late death. Our study demonstrates a high level of functional redundancy in the ability of different lymphocyte subsets to provide interferon gamma (IFN-γ), a critical cytokine in *Salmonella* immunity. Whereas early control of the infection was entirely dependent on IFN-γ, but not on any particular lymphocyte subset, clearance of the infection critically required CD4⁺ T cells, but appeared to be independent of IFN-γ. These data reinforce the idea of a bimodal immune response against *Salmonella* – an early T cell-independent but IFN-γ-dependent phase and a late T cell-dependent phase that may be IFN-γ-independent..
INTRODUCTION

The intracellular pathogen \textit{Salmonella enterica} is the causative agent of typhoid fever and gastroenteritis, conditions with considerable global human morbidity and mortality (1, 2). Infections with non-typhoidal strains of \textit{Salmonella enterica} (NTS) are also a major cause of fatal systemic bacteremias in HIV$^+$ individuals in Sub-Saharan Africa (3, 4), amongst which ST313 \textit{S. Typhimurium} pathovars are the most common isolates (5, 6). Vaccine development for invasive NTS (iNTS) is currently being evaluated using multiple approaches (3).

The cytokine interferon gamma (IFN-\(\gamma\)) plays a critical role in the control of experimental and clinical Salmonellosis (7) with genetic deficiencies in IFN-\(\gamma\), IFN-\(\gamma\)-inducing cytokines, its receptor or downstream response genes associated with increased susceptibility to severe \textit{Salmonella} infections (7-10). HIV infections are characterized by a gradual decline in CD4$^+$ T cells, the cell-type believed to be a prime producer of IFN-\(\gamma\) in response to \textit{Salmonella} infections (1). In order to develop therapies and vaccines against iNTS that are effective in T cell deficient HIV$^+$ individuals, including those on antiretroviral therapy, it is useful to identify CD4-independent mechanisms of \textit{Salmonella} immunity.

To study mechanisms of mammalian host resistance to Salmonellosis, the murine model for typhoid fever has been widely used and has been instrumental to advancing our understanding of immunity against \textit{Salmonella enterica} (1, 11). Despite efforts by many investigators, the role of individual immune cell subsets and their contributions to the control and clearance of the infection remains largely unresolved or confused. Conflicting reports in the literature about the roles of lymphocyte subsets in control of \textit{Salmonella} infections may have been due to discrepancies in infection strategies and \textit{Salmonella} strains, the use of different genetic backgrounds of the murine host, and a lack of reliable models for some lymphocyte deficiencies. Although previous studies have demonstrated crucial roles for both CD4$^+$ T cells and IFN-\(\gamma\) in anti-\textit{Salmonella} immunity (7, 12, 13), until recently it was not
clear whether these deficiencies are causally linked. We have recently shown that the production of IFN-γ by NK cells or memory CD8+ T cells in the absence of all other IFN-γ-producing lymphocytes is an important contributor to early host-protection (13-15). These results indicated an inherent capacity of non-CD4 immune cells to contribute to anti-
Salmonella immunity.

The present study was therefore designed to systematically investigate the cellular requirements for immunity against Salmonella. We used a low dose infection model with an attenuated S. Typhimurium strain (13, 14) carrying a deletion mutation in the aromatic pathway that was modeled on the aroA deletion mutant of S. Typhimurium SL1344, known as SL3261 (16). To focus on the mechanisms involved in clearance from systemic organs and to ensure high reproducibility between experiments, an intravenous infection was used. Although the natural way of infection with Salmonella is through the fecal-oral route, the final outcome of the infection with an attenuated strain is largely independent of the infection route (11, 12, 17).
MATERIALS AND METHODS

Mice

C57BL/6, IFN-γ−/−, Rag1/Jε−/−, Rag2γc−/−, IAE−/−, μMT, Kb−/−Db−/−, CD1d−/−, β2m−/−, IL-15−/−, GK1.5Tg, 2.43Tg and (GK1.5 × 2.43Tg)F1 mice were bred and maintained at The University of Melbourne. Phenotypic characteristics of each mouse strain are included in the results section. All animal experiments were approved by The University of Melbourne Animal Ethics Committee and were conducted in accordance with the Prevention of Cruelty to Animals Act (1986) and the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Scientific Purposes (1997).

Bacterial strains and infection

Salmonella enterica serovar Typhimurium BRD509 was grown statically at 37º C in Luria Bertani (LB) broth for 16 - 18 hrs, diluted in PBS and 200 cfu were injected into the lateral tail vein in a volume of 200 μl. The number of replicating bacteria was determined by homogenizing organs from infected mice and culture on Luria-Bertani (LB) agar plates supplemented with 25 μg/ml streptomycin. BRD509 was thought to be a mutant with deletions in aroA and aroD (18). We recently sequenced the genome of BRD509 and found aroD to be intact (data not shown). The strain remains aromatic compound-dependent through mutation of aroA (16).

Measurement of serum cytokine levels

Levels of IFN-γ, TNF, IL-6, IL-12p70, IL-10 and MCP-1 in mouse sera were analyzed using the BD™ Cytometric Bead Array (CBA) Mouse Inflammation Kit (BD Biosciences) according to the manufacturer’s instructions.
Data analysis

Statistical analysis was performed using GraphPad Prism version 5.0, (GraphPad software, CA).
RESULTS

Multiple lymphocyte subsets contribute to control of S. Typhimurium infections in mice

A total of 13 different mouse strains lacking one or more lymphocyte populations or cytokines were studied to determine the relative contribution of different immune cells and factors to BRD509-immunity (Fig. 1A). Transgenic mice constitutively expressing antibodies against CD4 (GK1.5Tg), CD8 (2.43Tg) or both (GK1.5Tg × 2.43Tg)F1 (19-21) enabled the study of the role of CD4+, CD8+ and double negative (DN) T cells in the experimental disease. These mice have a permanent absence of peripheral CD4+ and/or CD8+ T cells (20) and lack the residual Th cells present in CD4−/− mice (22-24), MHC class-II restricted CD8+ T cells and CD1d restricted CD4+ T cells in MHC-II−/− mice (25, 26). The use of mice lacking major histocompatibility complexes I or II and therefore all mature CD8+ T cells (β2m−/−), ‘classical’ CD8+ T cells only (Kb−/−Db−/−) or all mature CD4+ T cells (IAE−/−) not only helped to verify the results obtained in mice with transgenic T cell depletion but also shed light on the requirement of antigen presentation for the control and clearance of S. Typhimurium infections in mice. Additionally, the use of mice lacking the immunoglobulin µ chain (μMT) allowed us to study the contributions of B cells (27). The role of NKT cells was investigated through the infection of CD1d−/− mice (28). As classical NK cells and memory CD8+ T cells rely on interleukin 15 to develop and mature, the use of IL-15−/− mice (29) enabled us to investigate the role of these cells in Salmonella-immunity. Furthermore, we included genetic knockout mice that either lacked all B and T cells (Rag1/Jc−/−) (30), all lymphocytes (Rag2−/−γc−/−) (31) or the important effector cytokine IFN-γ (IFN-γ−/−) (32). All strains used (Fig. 1A) were on a susceptible Ity5 C57BL/6 background. Infected mice, representing all 13 mouse strains, were assessed for the number of viable bacteria in spleen (Fig. 1B) and liver (Fig. 1C) at different time points after infection. The analysis yielded four distinct outcomes – early death, late death, chronic infection and clearance (Fig. 1B, C).
Overall, the infection of spleen and liver showed a similar pattern with clearance occurring slightly faster in the liver. Consistent with previous reports (11, 12, 17), i.v. S. Typhimurium BRD509 infections, like infections with other aro-dependent strains (e.g. SL3261 (16)) followed a typical pattern: after an initial increase in bacterial numbers, a ‘plateau phase’ was observed in C57BL/6 mice, which subsequently cleared the infection within 7 to 12 weeks (1). Also consistent with previous reports (33-35), mice deficient in B cells (μMT) or NKT cells (CD1d<sup>-/-</sup>) cleared the infection in the same time frame as C57BL/6 mice, indicating that B cells and NKT cells are either not required for clearance of the bacteria or their absence can be compensated for by the remaining cell subsets.

All strains deficient in CD8<sup>+</sup> T cells (β<sub>2</sub>m<sup>-/-</sup>, Kb<sup>-/-</sup>Db<sup>-/-</sup>, 2.43Tg) cleared the infection indistinguishably from C57BL/6 mice implying that the absence of CD8<sup>+</sup> T cells alone does not inhibit the ability of the murine host to clear the bacteria (Fig. 1 B, C). Consistent with studies using class II (Aβ<sup>-/-</sup>) knockout mice (12) or injections of antibody against CD4 (36-38), data reported here show that GK1.5Tg and IAE<sup>-/-</sup> (MHC class II deficient) mice, which were CD4<sup>+</sup> T cell deficient, developed a non-lethal chronic infection. GK1.5Tg mice displayed stable bacterial levels for at least 21 weeks (data shown for up to week 12). In contrast, mice deficient in CD4<sup>+</sup> and CD8<sup>+</sup> T cells (GK1.5Tg × 2.43Tg)<sub>F1</sub> harbored approximately one hundred fold more bacteria in spleen and liver at 12 weeks after infection compared to GK1.5Tg mice (Fig. 1 B, C) and eventually became moribund supporting the idea that in the absence of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells can contribute to the control of the infection (‘T cell dependent phase’). (GK1.5Tg × 2.43Tg)<sub>F1</sub> still contain double negative (DN, CD4<sup>-</sup>CD8<sup>-</sup>) T cells and B cells. To determine the role of DN T cells and T cell-independent antibody responses (39), Rag1/Je<sup>-/-</sup> mice were used. Rag1/Je<sup>-/-</sup> mice contained higher numbers of Salmonella in spleen and liver at later time points, compared to (GK1.5Tg × 2.43Tg)<sub>F1</sub> and became moribund earlier; only
30% of Rag1/Je-/- mice were not moribund at week 12 p.i. compared to 70% for GK1.5Tg × 2.43Tg, suggesting a role for DN T cells and/or T cell-independent antibody responses in the late control of the infection. Rag1/Je-/- mice were able to control the infection for the first 4 weeks and could initiate a ‘plateau phase’ similar to C57BL/6 mice, whereas Rag2-/-γc-/- mice, that are deficient in B, T and NK cells became moribund within 4 weeks, which is remarkably similar to IFN-γ-/- mice. This data supported our previous results that NK cells in the absence of other lymphocytes can provide sufficient IFN-γ during the early phase of infection to initiate the ‘plateau phase’ and to control bacterial growth (14) (‘T cell independent phase’). However, in the presence of other lymphocytes, NK cells and memory CD8+ T cells seem to be redundant because IL-15-/- mice, which have severe reductions in both cell types but contain normal numbers of B and other T cells, also cleared the infection similar to C57BL/6 mice. Taken together these data reinforced the critical requirement for lymphocyte produced IFN-γ during early stages of the infection and suggested that CD8+ T cells, DN T cells and potentially also T cell-independent B cell responses (39) contribute to late control of S. Typhimurium infection in mice in the absence of CD4+ T cells.

IFN-γ as a correlate for bacterial numbers rather than a mediator of clearance during late stages of the infection

Since IFN-γ is crucial for controlling initial Salmonella growth and IFN-γ-/- mice become rapidly moribund and develop high bacterial numbers (Fig. 1 B, C) (7), the contribution of individual lymphocyte subsets to the production of IFN-γ, measured by serum levels of the cytokine, was studied in the different mouse lines, over time. All T cell subsets (CD4+, CD8+ and DN) responded to polyclonal restimulation and contributed to IFN-γ secretion proportional to their abundance (data not shown).
Like bacterial numbers in spleen and liver, the serum level of IFN-γ followed a 4-phase pattern. As expected, mice deficient in IFN-γ (IFN-γ−−) or all lymphocytes (Rag2−−γc−−) displayed either undetectable, or significant lower amounts of IFN-γ in their serum (Fig. 2 A). In contrast, all strains that cleared the infection followed a similar trend. The levels of IFN-γ in the serum peaked around three weeks after infection, at a similar level to that seen in C57BL/6 mice, and returned to levels below the detection limit by seven weeks (Fig. 2 B), suggesting that the absence of individual IFN-γ producing lymphocyte subsets can be compensated for by the remaining cell subsets. GK1.5, (GK1.5Tg × 2.43Tg)f1 and IAE−− mice, that either developed a chronic infection with bacteria or were unable to control the infection at late stages during the time course, had near constant serum levels of IFN-γ throughout the later phase of the infection, which was similar in magnitude to the peak level of IFN-γ in C57BL/6 mice (Fig. 2 C, D). Rag1/Je−− mice that succumbed to the infection between 7 and 12 weeks had an even higher serum level of IFN-γ, indicating that NK cells could compensate for the loss of other IFN-γ-producing cell types, at the total IFN-γ level (Fig. 2 D). Although most cytokines act locally, where high release of cytokines from a small number of cells may be more effective than increased circulating levels of the cytokine (40, 41), the serum IFN-γ levels suggested, that although IFN-γ is absolutely crucial for the initial control of the infection, it may not be the major factor which drives clearance of Salmonella Typhimurium in the mouse model.

In order to investigate whether other cytokines linked to inflammation, T cell activation and cell recruitment to sites of infection were also differentially produced during S. Typhimurium infections in immunocompromised mice, the levels of key cytokines were analyzed in the sera of infected mice over time. Similar to IFN-γ levels, the amount of monocyte chemotactic protein-1 (MCP-1, also known as CCL2), a cytokine required for the recruitment of monocytes, memory T cells and dendritic cells to sites of infections, peaked at 2 to 3 weeks
after infection in almost all mouse strains and fell below the detection limit by week 7 to 12 (Fig. 2 F). Consistent with the lack of clearance of bacteria in IFN-γ−/−, Rag2−/−γc−/− and Rag1/Je−/− mice, these animals all displayed high levels of MCP-1 (Fig. 2 E, H). Other acute phase cytokines, such as IL-6 and TNF-α, mirrored the bacterial load in mice that fail to clear the infection and increased rapidly in mice that became moribund early in the infection and more gradually in mice that developed a chronic infection or were unable to sustain control of the infection at later stages (Fig. 2 I-P). These results suggested that the serum levels of certain inflammatory cytokines, including IFN-γ, mirror bacterial numbers in systemic organs. IL-12p70 was virtually undetectable in all strains (Fig. 2 Q-T) and the anti-inflammatory cytokine IL-10 was only produced in large amounts immediately before Rag2γc−/− and IFN-γ−/− mice succumbed to the infection possibly indicating an attempt to counter excessive inflammation and the risk of endotoxic shock (Fig. 2 U-X).
DISCUSSION

In this study the role of lymphocyte subsets in immunity to Salmonella Typhimurium was systematically analyzed using a standardized infection model that combines previously investigated deficiencies with new knockout and transgenic mouse strains. Using 13 different mouse strains we show that sub-lethal infections with Salmonella Typhimurium in innately susceptible mice deficient in one or more lymphocyte subsets lead to four distinct outcomes - clearance, chronic infection, late death or early death. Whereas the early control of the infection is T cell independent but crucially dependent on IFN-γ, the final clearance stage appeared to be T cell dependent but may be IFN-γ independent.

The crucial role of IFN-γ for the control of attenuated S. Typhimurium during the early stages of the infection is in line with previous studies (7, 12, 42). Also in support of earlier research, the study presented here shows that CD4+ T cells are critically required for final clearance (12, 36). Furthermore the analysis confirmed that T cells are not required for the initiation of the ‘plateau phase’ (43, 44) and that NK T cells (35) and B cells (33, 34) are not essential for clearance of a primary infection. Important new findings from this comprehensive study include data showing: 1) that the IFN-γ that is required for early control of Salmonella may come from a variety of cell types and that NK cells and memory CD8+ T cells alone are sufficient to produce enough IFN-γ to enable control of the initial exponential growth of the bacteria (13, 14); 2) in the absence of CD4+ T cells, CD8+ and DN T cells contribute to control of S. Typhimurium and prevent mice from becoming moribund due to high bacterial numbers. Deficiency of classical, non-classical or all CD8+ T cell alone has no impact on the outcome of the infection; and 3) IFN-γ levels at later stages of the infection appear to be a correlate of bacterial burden rather than a mediator of clearance.

Several studies have focused on the role and the cellular sources of IFN-γ during S. enterica infections (7, 42) and clinical trials have investigated the feasibility of IFN-γ administration...
to treat infections with *S. Typhimurium* and other intracellular bacteria (45-47), but the results were mostly discouraging. Based on the observations that IFN-γ is integral to control of early infection and that the number of IFN-γ secreting T cells is likely highest just before bacterial clearance commences (12, 38, 48), it has been assumed that IFN-γ must be important throughout the course of the infection (49). However, the literature shows that administration of anti-IFN-γ antibodies at later stages of primary infections did not influence the final clearance of an attenuated *S. Typhimurium* strain (50). In the study reported here, GK1.5Tg and *IAE*−/− mice fail to clear the infection, but have wild type serum levels of IFN-γ and comparable numbers of IFN-γ producing T cells (as measured by *ex vivo* anti-CD3 re-stimulation; data not shown) throughout the entire observation period of 12 weeks.

Furthermore, *Rag1*/*Je*−/− mice that were unable to control the infection between seven and 12 weeks produced large amounts of IFN-γ in late stages of the infection, yet nevertheless failed to clear and control the infection. This data suggests that IFN-γ levels may be a correlate of bacterial numbers rather than as a mediator of clearance during late stages of the infection and that the mechanisms by which CD4+ T cells mediate clearance of *S. Typhimurium* may be independent of IFN-γ. Furthermore, these results imply that cognate stimulation and clonal expansion of specific CD4+ T cells (51) may be uncoupled from IFN-γ production and that other CD4+ T cell-mediated effector mechanisms probably contribute to bacterial clearance (50). Interestingly, very similar observations have recently also been reported during murine infections with *Mycobacterium tuberculosis* (52-55). Nevertheless, to conclusively answer what other effector mechanisms contribute or are necessary for bacterial clearance the development of a mouse model for the selective absence of IFN-γ in CD4+ T cells will be necessary.

Our data also suggests that mice deficient in CD4+ T cells can compensate for the loss of IFN-γ producing T cells by enhanced IFN-γ production by CD8+ T cells and partly by DN T
cells. Therefore it is possible that the increased IFN-γ production by CD8+ T cells prevents
CD4+ T cell-deficient mice from succumbing to infection, even though no clearance is
achieved and a chronic infection is established. Although very high local cytokine
concentrations from a small number of cells may be more efficient than a high average level
of the cytokine in the serum (40, 41), the data presented here raise the very interesting
question as to whether there might be qualitative differences in IFN-γ produced by different T
cell subsets. It appears that during Salmonella infection CD4+ T cell produced IFN-γ is more
important than CD8+ T cell produced IFN-γ which in turn is more important than DN T cell
produced IFN-γ. Such differences might reflect either the kinetics of cytokine expression
and/or very local levels. Although it is well known that different T cell subsets display unique
cytokine profiles under different circumstances (56, 57), no qualitative differences in IFN-γ
produced by one subset or the other have been reported so far.

But how else might DN T cells contribute to immunity against attenuated S. Typhimurium?
In spleen and liver of C57BL/6 mice DN T cells typically consist of approximately 50 % γδ
TCR+ and 50 % αβ TCR+ T cells. Apart from acting as conventional adaptive T cells
recognizing their cognate antigen, particularly γδ TCR DN T cells have also been shown to
exert innate functions, including potent production of IFN-γ independently of cognate
interactions. Whereas T cells expressing the αβ T cell receptor are absolutely indispensable
for clearance of an attenuated Salmonella infection, the role of cells expressing the γδ T cell
receptor is less clear. Antibody-mediated depletion of T cells either expressing the αβ T cell
receptor or the γδ T cell receptor in mice infected with Salmonella decreased the lethal dose,
suggesting that both T cell subsets are important in controlling a S. Typhimurium infection
(58). Furthermore infection of gene-targeted mice deficient in either αβ TCR+ or γδ TCR+
indicated a contribution but not a sufficient role of γδ TCR+ T cells to acquired immunity
against Salmonella (17). These results indicate a significant yet minor role for DN T cells in
Salmonella immunity and are consistent with our results that demonstrate a much greater difference in systemic bacterial loads between B6.GK1.5Tg vs. (B6.GK1.5 × B6.CMV2.43)F1 compared to the difference between (B6.GK1.5 × B6.CMV2.43)F1 vs. Rag1/Jc−/− mice. However, whether this is simply due to the greater abundance of CD8+ T cells compared to DN T cells can only be conclusively answered once a mouse model for the selective absence of DN T cells becomes available.

This study leaves the question as to what role CD4+ T cells really play in clearance of the infection. Only a few Salmonella T cell epitopes have been identified to date (59-62) and IFN-γ production in response to these epitopes was considerably lower than after polyclonal restimulation; moreover, adoptive transfer of these cells failed to protect mice from infection (60, 62) and late administration of anti IFN-γ antibodies did not affect bacterial clearance (50). It is possible that the IFN-γ-independent, but antigen-specific, clearance of Salmonella by CD4+ T cells could be due to CD40L-dependent T cell help, i.e. like that provided by CD4+ T cells to immunoglobulin class switching by B cells (63, 64). This hypothesis is supported by the fact that CD28−/− mice produce very low levels of Salmonella-specific IgG1, IgG2a or IgG2b antibodies (65, 66). Although this study and others showed that mice deficient in B cells clear a primary infection with an attenuated strain just as well as C57BL/6 mice (33, 34), protection against a secondary lethal infection with wild-type Salmonella critically depends on the presence of B cells (33, 34, 67) but not antibodies (68). Interestingly B cells can be infected by Salmonella in vivo and in vitro (69, 70) and B cells can present Salmonella antigens to T cells (69). Furthermore, CD4+ T cells derived from B cell deficient mice (Igh-6−/−) show reduced secretion of IL-2 and IFN-γ (34). Therefore it is conceivable that Salmonella-specific CD4+ T cells may be required for the initiation of a B cell mediated response against Salmonella and B cells may influence Salmonella-specific T cell responses (68). Such a model does not explain why B cell-deficient mice are capable of clearing a...
primary infection if the same B-T cell interactions are an important component of bacterial clearance. Alternatively, CD4⁺ T cell produced IFN-γ may also be crucial for the development of efficient anti-Salmonella CD8⁺ T cell responses. This idea was recently supported by Green and colleagues who suggested a critical requirement of CD4⁺ T cell produced IFN-γ for a quality CD8⁺ T cell response during murine M. tuberculosis infection (53). Finally, there may be a role for T cell-independent antibody responses in infection clearance (39).

Collectively, the results reported here not only support the feasibility of further exploring non-CD4⁺ T cell-mediated mechanisms in immunity against Salmonella Typhimurium but also call for an enhanced effort to delineate the mechanisms by which CD4⁺ T cells really contribute to the clearance of Salmonella (and other intracellular pathogens). The comprehensive and systematic infection model presented here might assist in these efforts and could contribute to the development of a vaccine or immunomodulatory approach against invasive Salmonella disease.


59. Lo, W. F., H. Ong, E. S. Metcalf, and M. J. Soloski. 1999. T cell responses to gram-negative intracellular bacterial pathogens: A role for CD8(+) T cells in...
immunity to Salmonella infection and the involvement of MHC class Ib molecules. Journal of Immunology 162:5398-5406.


FIGURE LEGENDS

**Figure 1:** Comprehensive model to assess the role of different immune cell subsets and IFN-γ production in immunity to attenuated Salmonella Typhimurium BRD509 infection. (A) Schematic representation of the absence or presence of different lymphocyte populations in all mouse strains used for the infection model. White fields indicate absence of a particular population; grey fields indicate the presence of the cell type. All strains used were of a ‘susceptible’ C57BL/6 background. (B, C) Mice were infected intravenously with 200 cfu S. Typhimurium BRD509. At indicated weeks post infection, groups of at least 5 mice were killed and viable bacteria in the spleen (B) and liver (C) were assessed. Infection with Salmonella Typhimurium BRD509 follows two distinct phases. Early control of infection and initiation of the ‘plateau phase’ (up to 4 weeks) is dependent on IFN-γ and lymphocytes but independent of T, B and NKT cells (a). Final clearance of bacteria is dependent on T cells but largely independent of serum IFN-γ levels (b). Based on the mouse strains used, four distinct outcomes of the infection can be distinguished: early death (1), late death (2), chronic infection (3) and clearance (4). Dotted lines indicate cut off for individual detection limits. Data representative of mean ± SEM of at least five to ten mice per time-point and pooled from up to three independent experiments. Parts of B week 3 were previously published in (17) and are reproduced with permission of the Proceedings of the National Academy of Sciences (PNAS). Statistical information for B and C is provided in Supplemental Table 1.

**Figure 2:** Analysis of inflammatory cytokines. 13 different genetically modified mouse strains of a C57BL/6 background were infected intravenously with 200 cfu S. Typhimurium BRD509 and culled at weeks 1, 2, 3, (4), 5, 7 and 12 after infection. Heart blood was taken and sera of all mice were analyzed for IFN-γ (A-D), MCP-1 (E-H), IL-10 (I-L), IL-6 (M-P), TNF (Q-T) and IL-12p70 (U-X) by cytometric bead array (CBA). Data representative of
mean ± SEM of at least five to ten mice per time-point and pooled from up to three
independent experiments. Statistical information for all results is provided in Supplemental
Tables 2 and 3.
CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure 1

A

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B

1. IFN-γ^- dependent/T cell independent phase
2. likely IFN-γ^- independent/T cell dependent phase

C

1. Early death
2. Late death
3. Chronic infection
4. Clearance
IFN-γ

A: Early death
B: Clearance
C: Chronic infection
D: Late death

IFN-γ−/−
Rag2−/−
γc−/− (GK1.5Tg × 2.43Tg)
F1
C57BL/6
μMT
Rag1/Jc
−/−
GK1.5Tg
IAE−/−
IL-15−/−
2.43Tg
Kb−/−
Db−/−
β2m−/−

Figure 2