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MHC class II antigen presentation by B cells in health and disease

Souwer, Y

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Chapter 3

Immune escape and dissemination of *Salmonella* by antigen-specific B lymphocytes

Yuri Souwer, Alexander Griekspoor, Chiara Martinoli, Andrea Chiavelli, Tineke Jorritsma, Maria Rescigno, Jacques Neefjes and S. Marieke van Ham

Y.S. and A.G. contributed equally to this work.

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Abstract

The bacterial pathogen *Salmonella* causes worldwide morbidity and mortality. A major route of host entry in the intestinal mucosa is via M cells, directly entering the B cell-rich Peyer's Patches. *Salmonella* can be taken up by antigen-specific B cells in a B cell receptor (BCR)-dependent fashion. We studied the contribution of B cells to the pathogenicity of *Salmonella typhimurium*. Upon phagocytosis of *Salmonella* by primary human B cells, *Salmonella* survives intracellularly in a dormant state that is actively maintained by the B cell. Subsequently, *Salmonella* is excreted by B cells, allowing infection of other cell types and reinitiation of replication. B cells may thus function as a transport vehicle for *Salmonella*. Indeed, adoptive transfer of *Salmonella*-specific B cells before oral infection of mice with *Salmonella* was required to disseminate *Salmonella* to the spleen. *Salmonella*-specific B cells thus function as survival niche and reservoir to contribute to systemic dissemination of *Salmonella* and represent the first example of a pathogen that uses cells of the adaptive immune system for spreading of infection.

Introduction

Salmonella enterica is a Gram-negative, enteric pathogen responsible for disease syndromes of significant morbidity and mortality (1). After oral uptake, the bacterium crosses the intestinal epithelium and enters the Peyer's patches via specialized antigen-sampling M cells (2) or via luminal capture by dendritic cells (3, 4). They are ultimately internalized by macrophages, dendritic cells, and neutrophils (5, 6). Entry into these cells is actively induced by the bacterium through an impressive array of effector proteins that orchestrate uptake by manipulating the host cellular machinery (7). *Salmonella* manipulate host cells upon infection in order to alter the actin cytoskeleton allowing phagosomal cup formation and entry of the relatively large pathogen into the host cell. Bacterial effector proteins are therefore introduced into the host cytosol through the *Salmonella* Type III Secretion System (TTSS). *Salmonella* can thus enter most cell types to form an intracellular vacuole called the *Salmonella*-containing vacuole (SCV). Here, another set of effectors is secreted into the host cytosol for vacuole maintenance and interference with the endosomal system to obtain nutrients and to prevent maturation and fusion with lysosomes (8, 9) by manipulating the Akt-AS160-Rab14 cascade and PAK4 (10). *Salmonella* replicates in an expanding SCV (11, 12) and escapes detection by the immune system (13, 14). Although *Salmonella* replicates in the phagosome, it remains unclear how the bacteria are released from the infected cell. Obvious mechanisms would involve apoptosis or necrosis of the infected cell, but such is not established.

When *Salmonella* has passed the intestinal epithelium, it spreads via mesenteric lymph nodes to liver, bone marrow and spleen where replication continues (15). How *Salmonella* reaches these organs is unclear. So far, dendritic cells, macrophages, neutrophils and CD18-expressing phagocytes have been implicated to be the target cells of *Salmonella* infection (4, 16). Similar to HIV (reviewed in (17)), dendritic cells may act as pathogen carriers for more systemic spreading of the infection. Macrophages, dendritic cells and neutrophils however, exhibit efficient bactericidal mechanisms (6, 18) that render these cells less favorite as vehicles for bacterial dissemination. *Salmonella* is able to enter and survive within human B cell lines (19, 20). In mice, infected B cells are able to present *Salmonella* antigens to CD4⁺ T cells (21). We recently showed that primary human B cells are able to internalize *Salmonella* after recognition by the B cell receptor (BCR) and subsequently present bacterial antigens to *Salmonella*-specific CD4⁺ T cells (22).

Here we show how primary antigen-specific B cells are used as transport vehicle for spreading within the host. We show that *Salmonella* utilizes the specificity of the immune system after uptake by antigen-specific B cells to survive intracellularly in a dormant state that is actively maintained by the B cell. Ultimately, *Salmonella* is excreted by the B cell followed by reinfection and replication in other cell types. Adoptive transfer of B cells with transgenic BCRs that specifically recognize hen egg lysozyme (HEL)-expressing *Salmonella* showed that *Salmonella*-specific B cells contribute to the *in vivo* dissemination of *Salmonella* in mice after oral administration of the bacteria. The antigen-specific B cells thus act as antigen-specific reservoirs or transport vehicles to release *Salmonella* at distant sites for further infection. These data provide the first example of the use of the adaptive immune system by bacterial pathogens for spreading infection in a situation analogous to the involvement of dendritic cells in the spreading of HIV.

Materials en methods

Mice

C57BL/6 mice (6–8 weeks old) were purchased from Harlan (Udine, Italy). BCR-HEL VDJ ki mice (a kind gift of Dr. J. Cyster, University of California, San Francisco) were bred under specific pathogen-free conditions at Charles River Laboratories. All experiments were performed in accordance with the guidelines established in the Principles of Laboratory Animal Care (directive 86/609/EEC).

Evaluation of Salmonella spreading in vivo.

CD43- naïve B cells were purified from spleens of BCR-HEL VDJ ki mice with CD43 (Ly-48) Microbeads (Miltenyi Biotec, Bologna, Italy) according to the manufacturer's instructions. 10^6 - 2×10^5 cells were injected intravenously into C57BL/6 mice (14 mice per group) one day before oral infections. For survival experiments, mice received 10^5 CFUs of HEL surface-expressing *S. typhimurium* SL1344, and survival was monitored daily. For evaluation of splenic colonization, mice received 10^5 CFUs of bacteria. Sixteen hours after infection, spleens were collected and processed; a fixed number of cells was lysed with 0.5% sodium-deoxycholate and plated onto TB-agar plates for CFU counting 12 hr later.

Antibodies and bacterial strains

mAb anti-human IgM (MH15, Sanquin, Amsterdam, The Netherlands) was mixed with rat anti-mouse IgG1 antibody (RM161.1, Sanquin) and mAb anti-*S. typhimurium* LPS (1E6, Biodesign International, Kennebunk, ME) to generate BCR-LPS tetrameric antibody complexes. Fluorescent secondary antibodies and Texas Red-phalloidin were from Molecular Probes (Leiden, The Netherlands). GFP-*S. typhimurium* SL1344 has been described (23). The *S. typhimurium* strain 14028 containing the lux operon of *P. luminescens* (luxCDABE) was a kind gift from S. Vesterlund (24) and K. Neilson. Exponentially grown bacteria were washed with PBS, incubated with BCR-LPS tetrameric antibody complexes for 30 min at RT, and washed twice to remove unbound antibodies. Surface HEL-expressing *S. typhimurium* SL1344 was generated by electroporating bacteria with a pVUB4 vector (kindly provided by P. Cornelis, Flanders Institute for Biotechnology, Brussels, Belgium (25)) in which inactive HEL-encoding gene was cloned in frame with the one encoding for OprI protein from *P. aeruginosa* under the control of LacZ promoter. HEL expression was induced by the addition of 1 mmol/L isopropyl-L-thio-B-D-galactopyranoside to exponentially growing bacteria (26).

Lymphocyte isolation, infections and cell lines

Isolation of human B cells from peripheral blood and culturing of the Ramos B cell line and NIH3T3 fibroblasts expressing human CD40L (3T3-CD40L) have been described (22). B lymphocytes with viable uncoated bacteria and Ramos cells with viable anti-IgM coated bacteria were incubated for 40 min at 37°C w/o antibiotics while tumbling. Next, cells were washed four times and cultured for 1h in media containing 100 µg/ml gentamycin (Invitrogen) to eliminate non-phagocytosed bacteria. Cells were cultured in RPMI 1640 medium with 5% FCS, p/s, 2 mM L-Glutamine, 50 µM 2-mercaptoethanol, 20 µg/ml human apo-transferrin ((Sigma-Aldrich) depleted for human IgG with prot-G sepharose) and 10 µg/ml Gentamycin.

Live cell imaging

Wide field microscopy was performed at 37°C using 6-well plates (coated with Poly-L Lysine) and a Zeiss Axiovert 200 M microscope equipped with a FluorArc fluorescence lamp, motorized scanning stage, 63x LD Achromplan objective; NA 0.75 and climate chamber. Images were acquired using a Zeiss AxioCam MRm Rev.2 CCD in combination with the manufacturer's AxioVision software. All experiments

presented were repeated several times on different days, and results were consistent and reproducible. Further image processing was performed using the ImageJ software package.

Intracellular survival and growth assays

Human primary B cells were incubated in parallel experiments with either GFP- or Lux-expressing *Salmonella* with 2 bacteria per cell. The percentage of living cells and GFP levels were determined using a FACS Calibur (Becton Dickinson). Bioluminescence was measured for 5s in a luminometer (Berthold). Bacterial growth was determined by dividing the relative bioluminescence signal by the relative number of GFP⁺ living B cells, resulting in the amount of light produced per bacteria containing B cell. For induction of apoptosis, cells were treated with 0.1 μ M Edelfosine (Biomol) (27).

Bacterial excretion assay

To visualize bacterial excretion, human primary B cells were incubated with uncoated GFP-*Salmonella* at 20 bacteria per cell, and followed using wide field microscopy in medium containing anti-LPS antibodies coupled to TexasRed. To quantify excretion, cells were stained with DAPI (Sigma-Aldrich) to exclude dead cells and anti-LPS coupled to APC and fixed with 3.7% formaldehyde before analysis using a LSR II (Becton Dickinson). For the increase in LPS levels, the initial level at time point 0 was set to 1. The percentage of excreted bacteria was calculated as the loss of GFP⁺/LPS⁻ B cells compared to time point 0. To discriminate between bacterium and B cell-induced excretion, cells were cultured in medium containing 10 μ g/ml tetracycline to arrest intracellular bacteria (bacteriostatic capacity was verified using lux-*Salmonella* in Ramos cells).

Statistical analysis

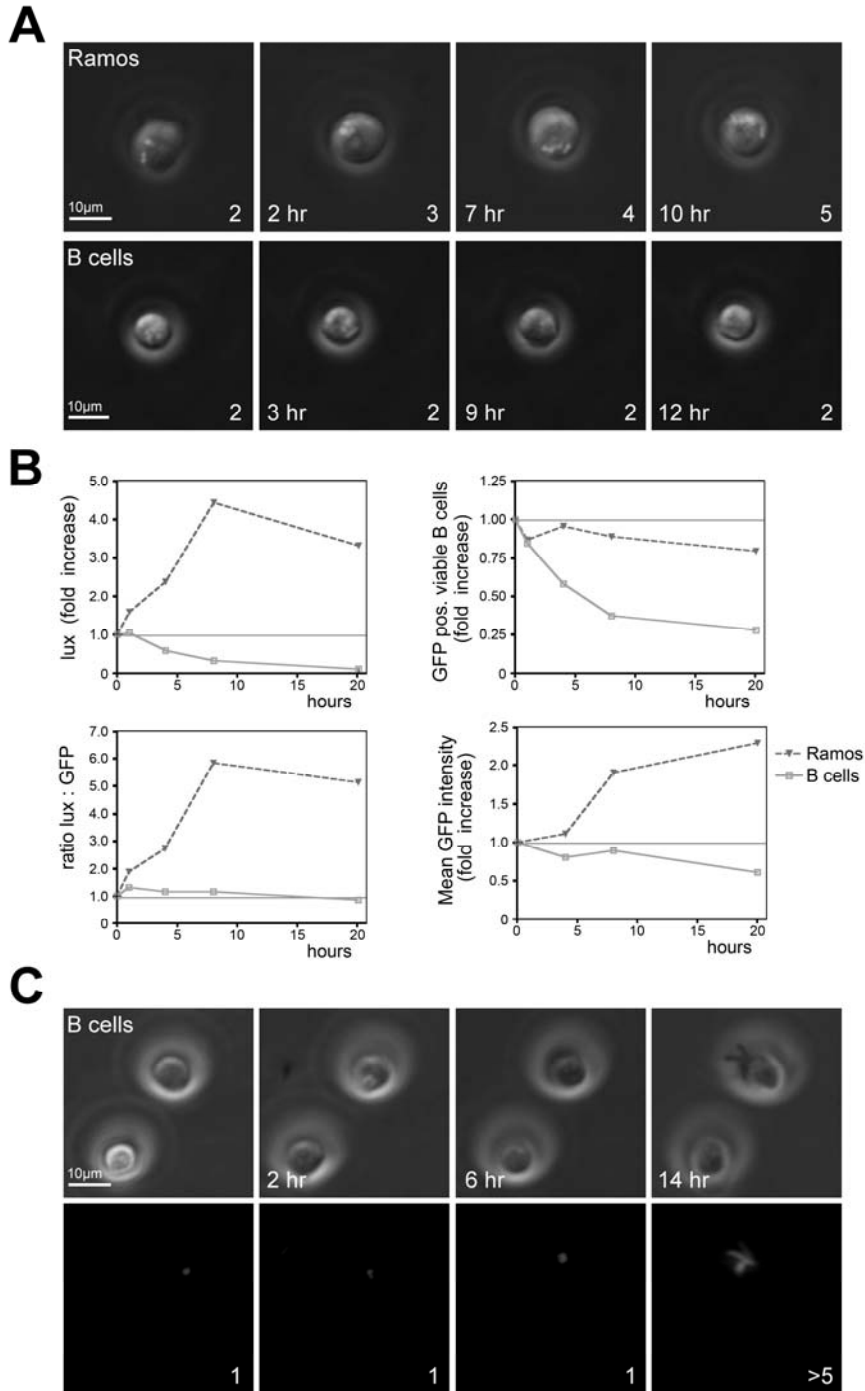
Kaplan-Meier plots and long-rank tests were used to assess survival differences of adoptively transferred mice after virulent *S. typhimurium* infection. Statistic calculations were performed by JMP 5.1 software (SAS, Cary, NC).

Results and Discussion

Primary human B cells form a survival niche for intracellular Salmonella

Recent data indicated that B cells from early vertebrates act as efficient phagocytes in contrast to mammalian B cells that do not show phagocytic behavior (28). We demonstrated however, that human B cells have not lost this phagocytic capacity but will only phagocytose particles or pathogens when first recognized by the BCR (22). The BCR acts as an antigen-specific receptor for *Salmonella*, and BCR crosslinking likely cooperates with bacterial effector proteins to facilitate uptake in B cells. Other mammalian cells are infected by *Salmonella* without any further involvement of such specific receptors. Phagocytosed *Salmonella* grows in many cell types, and can only be efficiently destroyed in specialized cells like macrophages and neutrophils in a process requiring the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system (5). We previously noticed survival of *Salmonella* in B cells after BCR-mediated internalization and now studied the fate of these phagocytosed *Salmonella* in more detail.

To study the fate of *Salmonella typhimurium* in B cells, time-lapse imaging of GFP-*Salmonella* containing B cells was performed with wide-field microscopy to limit phototoxicity. After incubation with bacteria and extensive washing, we observed replication of GFP-*Salmonella* in the Ramos B cells (Fig. 1A, *top panel* and supplemental movie 1), whereas *Salmonella* did not multiply in primary B cells (Fig. 1A, *bottom panel* and supplemental movie 2). To quantify these observations, we performed parallel experiments to compare GFP-*Salmonella* (detected by FACS analysis) with light producing lux-*Salmonella* (detected by luminometry). Light production by lux-*Salmonella* requires ATP and is thus a marker for bacterial viability (24). Figure 1B shows that anti-IgM coated GFP-*Salmonella* that have been phagocytosed by Ramos cells expanded intracellularly. Over a time course of 10h we observed a strong increase in lux activity (Fig. 1B, *top left panel*), while the number of GFP-*Salmonella* positive viable Ramos cells remained nearly constant (Fig. 1B, *top right panel*). The amount of light produced in GFP-*Salmonella* positive Ramos cells increased considerably (Fig. 1B, *bottom left panel*), indicating that the number of bacteria per Ramos cell increased over time. In accordance, the GFP signal per Ramos cell increased (Fig. 1B, *bottom right panel*). In contrast, lux activity sharply dropped over time when *Salmonella* was phagocytosed by primary human B cells (Fig. 1B, *top left panel*). This was not due to elimination of



Salmonella as the fraction of GFP-*Salmonella* containing B cells declined equally fast (Fig. 1B, top right panel). In fact, the amount of light produced per living GFP-*Salmonella* positive B cell remained constant during the course of the experiment (Fig. 1B, bottom left panel), showing that *Salmonella* remained viable in primary human B cells, albeit under conditions of inhibited proliferation. This confirms the wide-field microscopy data that showed that *Salmonella* does not replicate in primary B cells. Unlike specialized phagocytic immune cells like macrophages,

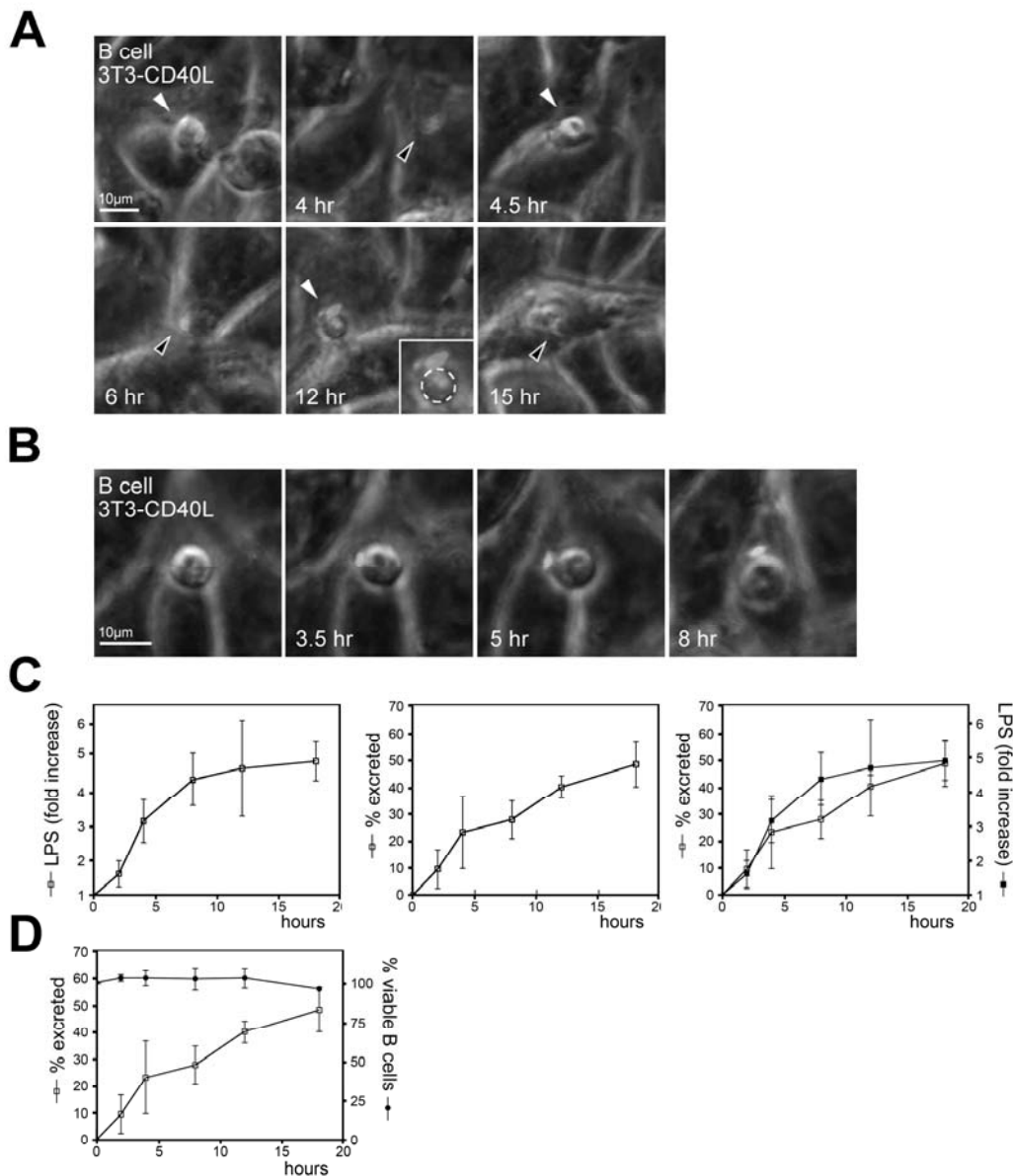
Figure 1. Primary human B cells form a survival niche for intracellular *Salmonella*.

(A) Widefield fluorescence microscopy of living Ramos and primary human B cells with phagocytosed GFP-expressing *Salmonella*. Bacteria were coated with anti-IgM mAbs to force BCR-specific recognition of the bacteria by the Ramos B cell line and to enhance the number of primary B cells that can phagocytose bacteria via their BCR. Depicted are GFP signals projected on the transmission image. Scalebar = 10 μ m. Number of bacteria in the visualized cell is given in the lower right corner. Lower left corner: time after *Salmonella* infection. Images are frames from supplemental movie 1 and 2. (B) Analysis of Ramos and primary human B cells incubated with living anti-IgM coated lux-expressing (*top left panel*) or GFP-expressing (*top right panel*) *Salmonella*. The ratio of lux over GFP shows the amount of light produced per GFP-*Salmonella* positive B cell (*bottom left panel*), indicating intracellular *Salmonella* viability. The mean fluorescence of the GFP positive population, set arbitrarily at 1 at the beginning of the experiment, shows that the GFP signal increases in Ramos B cells, whereas it decreased in primary human B cells (*bottom right panel*). A representative example of three independent experiments is shown. (C) B cells were infected with anti-BCR coated GFP-expressing *Salmonella* before exposure to Edelfosine to induce apoptosis. Cells were imaged over a 14h period. *Top panel*: transmission image, *bottom panel*: GFP-signal. Scalebar = 10 μ m. Images are frames from supplemental movie 3.

neutrophils, or B cells from early vertebrates (28), human B cells are apparently inefficient in producing the microbicidal conditions that are required to eliminate *Salmonella*. We next investigated whether primary B cells actively suppress *Salmonella* growth. We selectively induced apoptosis of B cells (without affecting *Salmonella*) and measured *Salmonella* replication. Intracellular replication of *Salmonella* was no longer suppressed 2h after induction of apoptosis in primary B cells with the alkyl-lysophospholipid Edelfosine (27) (Fig. 1C and supplemental movie 3), demonstrating that growth arrest of *Salmonella* requires viable primary B cells. These data suggest that primary human B cells, unlike human B cell lines, actively suppress multiplication of intracellular *Salmonella* within the SCV.

Salmonella is excreted by B cells and infects secondary host cells

The number of primary B cells that had initially phagocytosed *Salmonella* dropped over time (Fig. 1B, *top right panel*), suggesting that *Salmonella* may be released. To visualize the fate of phagocytosed *Salmonella* in B cells, primary human B cells infected with GFP-*Salmonella* were co-cultured on a monolayer of CD40L-expressing 3T3 cells and analyzed by time-lapse wide-field microscopy. In addition, CD40L will provide a pro-survival signal (29) enabling long term cell culture and imaging of primary B cells. Primary B cells that had phagocytosed GFP-*Salmonella* showed extensive invasive behavior by continuously moving under and over the



3T3-CD40L monolayer (Fig. 2A and supplemental movie 4). At later time points, some GFP-*Salmonella* appeared to be exocytosed from the B cell. To visualize this, GFP-*Salmonella* infected primary B cells were cultured in the presence of a low concentration of Texas-Red labeled anti-LPS mAb in the medium. GFP-*Salmonella* will attract and concentrate this antibody upon exposure to the medium. Figure 2B shows a B cell with phagocytosed GFP-*Salmonella* that becomes accessible for anti-LPS antibodies in the medium after 8 hr of culture (supplemental movie 5). *Salmonella* excretion from primary B cells was quantified using FACS by detecting GFP-*Salmonella* and LPS-positive B cells. Strong increase in cell surface exposed LPS on cells that were initially GFP-*Salmonella* positive and LPS negative was observed (Fig. 2C, left panel). This suggests that a large fraction of the phagocytosed *Salmonella* were exocytosed as in the example shown in Fig. 2B.

Figure 2. *Salmonella* is actively excreted by B cells and capable of infecting secondary host cells.

(A) Primary B cells having phagocytosed anti-BCR coated GFP-*Salmonella* on a monolayer of 3T3-CD40L fibroblasts were imaged using widefield fluorescence microscopy. Depicted is the GFP signal projected on the transmission image with images taken every 30 min. Scalebar = 10 μ m. Arrows indicate the B cell, white arrow: B cells moves op top of the monolayer, black arrow: B cells moves below the monolayer. Images are frames from supplemental movie 4. (B) Primary B cells having phagocytosed anti-BCR coated GFP-*Salmonella* on a monolayer of 3T3-CD40L fibroblasts were imaged using widefield fluorescence microscopy in the presence of TexasRed labeled anti-LPS mAbs. Depicted are GFP and Texas-Red signals projected on the transmission image. Scalebar = 10 μ m. Images are frames from supplemental movie 5. (C) Quantification of *Salmonella* secretion from B cells. Primary B cells were incubated with live uncoated GFP-*Salmonella*. Cells were stained with antibodies against LPS, fixed and analyzed using FACS. *Left panel*: increase in cell surface exposed LPS from bacteria exposed at the cell surface after initial uptake by B cells. *Middle panel*: percentage of B cells having excreted *Salmonella* as calculated from the percentage of B cells containing GFP-*Salmonella* followed in time. *Right panel*: left and middle panels are projected to illustrate that both processes show similar kinetics. Error bars represent SD from three independent experiments. (D) Primary B cells were incubated with live uncoated GFP-expressing *Salmonella* and followed for the time points indicated. The fraction of living B cells is plotted to demonstrate that loss of GFP-*Salmonella* positive B cells is not correlated with cell death.

Accordingly, the population of GFP-*Salmonella* positive/LPS negative B cells declined over time inferring increase in excretion (Fig. 2C, *middle panel*) with kinetics that were identical to the acquired LPS signal over time (Fig. 2C, *right panel*). GFP-*Salmonella* infection of primary B cells did not affect B cell viability or induced apoptosis (Fig. 2D). Note that during the first phase of excretion *Salmonella* was released, but remained associated to the B cells, hence the increased staining with the anti-LPS antibodies in the first 10h. The bacterium was later released from the B cell, leveling off further LPS labeling. Loss of the GFP-*Salmonella* signal from infected primary B cells increased over an 18h period in our experiments at which point more than 50% of the bacteria were released from B cells. Thus, a large proportion of phagocytosed *Salmonella* are secreted within 24 hrs of human primary B cell infection.

Excretion of Salmonella is a cell autonomous process

Does *Salmonella* actively participate to the process of excretion from B cells? So far, we used the antibiotic gentamycin in our experiments to kill extracellular *Salmonella* and prevent their overgrowth, as gentamycin does not affect intracellular *Salmonella* replication (as seen in Fig. 1A). Unlike gentamycin, the antibiotics tetracycline and erythromycin kill *Salmonella* intracellularly (30).

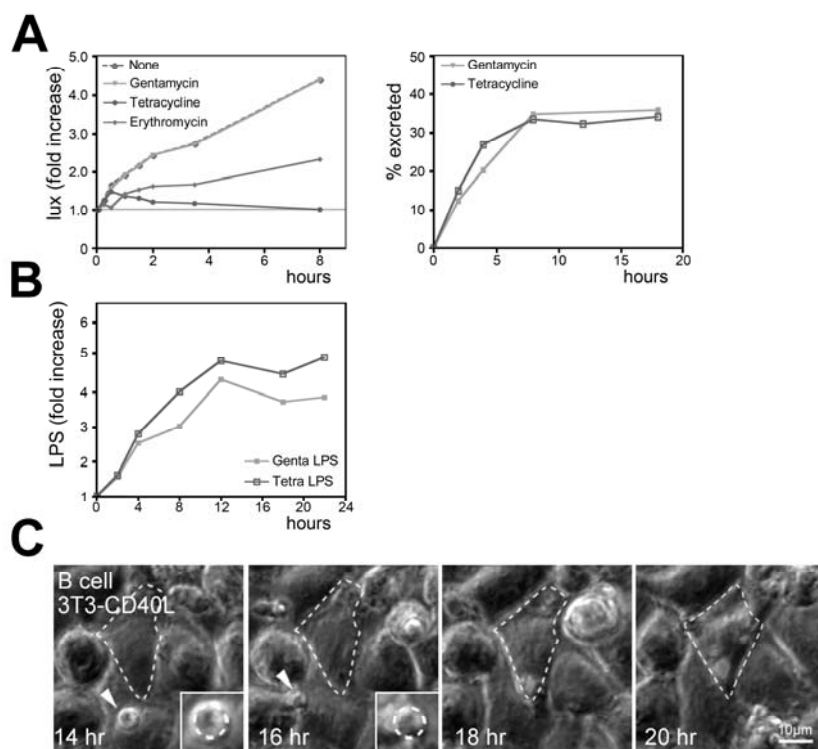


Figure 3. *Salmonella* is actively excreted by B cells and capable of infecting secondary host cells (A) *Left panel:* the effect of antibiotics on the growth of lux-*Salmonella* in Ramos B cells. *Right panel:* the same FACS analysis as in 2C was performed in presence of either Gentamycin or Tetracycline to discriminate between host and bacteria mediated excretion. (B) The same FACS analysis as in Fig. 2C was performed in presence of either Gentamycin or Tetracycline to discriminate between host versus bacterial-mediated excretion. Increase in cell surface LPS levels is similar in the presence of Gentamycin and Tetracycline, indicating that viable *Salmonella* are not required for excretion. (C) Primary B cells having phagocytosed anti-IgM coated GFP-*Salmonella* on a monolayer of 3T3-CD40L fibroblasts were imaged using widefield fluorescence microscopy for the times indicated. Imaging conditions are similar as in 2A. GFP-*Salmonella* is excreted from a primary B cell (white arrowhead), followed by infection of the 3T3-CD40L monolayer (outline of infected cell marked by a dashed line). Inset shows zoom-in on primary B cell excreting GFP-*Salmonella*. Images are frames from supplemental movie 6.

These antibiotics inhibited lux-*Salmonella* growth in Ramos cells (Fig. 3A, *left panel*) and no viable *Salmonella* bacteria were recovered in plating assays of infected B cells, in contrast to exposure to gentamycin (data not shown). *Salmonella* secretion by primary B cells was measured in the presence of either gentamycin or tetracycline. Tetracycline did not affect excretion of GFP-*Salmonella* from primary B cells, which occurred equally efficient as in the presence of gentamycin (Fig. 3A, *right panel*), indicating that *Salmonella* viability is not required for excretion. Likewise, a similar increase in cell surface LPS levels was observed in the presence

of tetracycline as with gentamycin (Fig. 3B). While *Salmonella* participates in uptake after capture by the BCR (22), excretion does not require viable *Salmonella*. Can *Salmonella* then infect other cells following transport by B cells to distant sites? We co-cultured primary human B cells containing phagocytosed GFP-*Salmonella* on a monolayer of 3T3-CD40L and followed post-excretion events using time-lapse wide-field microscopy. Figure 3C shows an example of a phagocytosed GFP-*Salmonella* that subsequently extruded from the primary B cell followed by infection of the fibroblast monolayer (supplemental movie 6). The released bacteria infected and replicated in the fibroblast monolayer, demonstrating that passage through primary B cell had not suppressed bacterial replication in an irreversible manner. These data suggest that *Salmonella* can use primary B cells as a reservoir or transport vehicle allowing it to escape immune attack and hitch-hike to distant locations.

Salmonella-specific B cells mediate spreading in acute in vivo infection.

Our observations imply that the availability of B cells with pathogen-specific BCRs would be advantageous for the spreading of infection as they may be used as *Salmonella* carriers. How *Salmonella* reaches the spleen is not clear. Possibly, *Salmonella* encounters specific B cells as they cross the intestinal epithelium via M cells directly located to gut-associated lymphoid tissue (GALT) sites where many B cells reside in Peyer's patches. Among preferred distant sites of persistent infection are the spleen and lymph nodes. *Salmonella* has been isolated from splenic macrophages and splenic B cells of orogastrically infected mice (20). *Salmonella* was thought to reach these locations after transport by neutrophils (16), a notion incompatible with the efficient bactericidal capacity of neutrophils (5). Also macrophages and DCs have been implicated, but also these cells have bactericidal capacities, like neutrophils. The possibility that *Salmonella* uses antigen-specific primary B cells as transport vehicle to distant sites in the host has not been considered. To test this option *in vivo*, we adoptively transferred C57BL/6 mice with increasing amounts of CD43- naïve B cells carrying a BCR specific for the HEL antigen. One day after transfer, mice were infected with a sublethal dose of surface HEL-expressing *Salmonella*, and survival was monitored daily. Transfer of 2×10^5 HEL-specific B cells increased C57BL/6 mortality after HEL-expressing *Salmonella* infection whereas 10^6 B cells protected (Fig. 4A). These results may be due to two opposite effects of the antigen-specific B cells; spreading of *Salmonella* and

production of antibodies against *Salmonella*, in which the balance of these activities determines survival of mice. To directly establish if HEL-specific B cells mediated systemic dissemination of HEL-expressing *Salmonella*, we investigated bacterial recovery from the spleen in the different experimental settings. *Salmonella* spread to the spleen only after adoptive transfer of antigen-specific B cells (Fig. 4B). Thus, antigen-specific B cells were required for *in vivo* dissemination of *Salmonella* to the spleen.

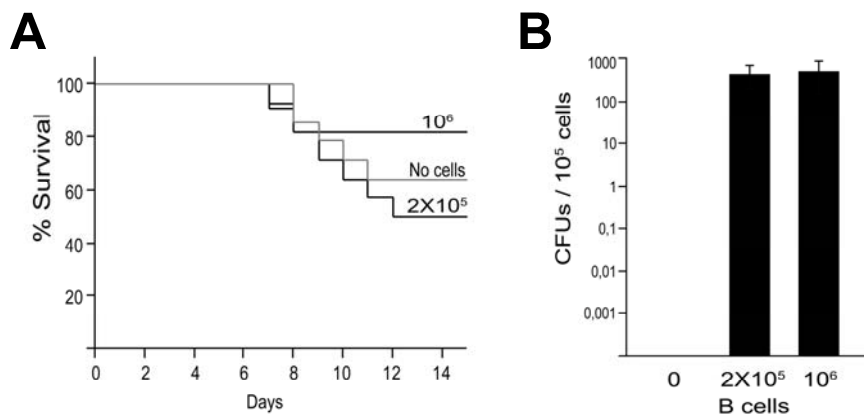


Figure 4. *Salmonella*-specific B cells form a survival niche and help *Salmonella* spreading through the body

(A) Kaplan-Meier survival curves of C57BL/6 mice left untreated or adoptively transferred with 2×10^5 , or 10^6 HEL-specific CD43- naïve B cells. Mice ($n=14$ /group) were orally infected with surface HEL-expressing *Salmonella* one day after transfer. Survival was monitored daily. (B) C57BL/6 mice were left untreated or adoptively transferred with 2×10^5 or 10^6 HEL-specific CD43- naïve B cells, as indicated. Mice were orally infected with surface HEL-expressing *Salmonella* one day after B cell transfer. Culturable splenic bacteria (CFU/ 10^5 cells) 16 hours after infection are shown ($n=3$ /group). Shown is the mean + SEM. One representative example of two independent experiments is shown.

Our data show that *Salmonella* misuses the specificity of the adaptive immune system. *Salmonella* may hide from the early innate immune defenses in antigen-specific B cells and may even create a sustained reservoir of infection by going through multiple rounds of uptake and excretion in B cell-rich areas like the Peyer's patches and spleen. In addition, *Salmonella* may hitch-hike in B cells to facilitate systemic spreading of the infection. Previously, it was shown that cells of the innate system and erythrocytes can be used by pathogens for systemic spreading of infection within immunocompetent hosts, like HIV (DCs) (31) and *P. falciparum* (erythrocytes) (32). The adaptive immune system has evolved to protect against infection, while simultaneously generating immunological memory to ensure a rapid

immune response against reinfection. We now show that *Salmonella* has adapted to this by using antigen-specific B cells for immune evasion and spreading to distant sites within the host. This is the first example of the use of cells from the adaptive immune system by bacteria to enable their dissemination.

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