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***Salmonella* disrupts lymph node architecture by TLR4-mediated suppression of homeostatic chemokines**

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Abstract

We report that infection of draining lymph nodes (DLNs) by *Salmonella typhimurium* results in the specific downregulation of the homeostatic chemokines CCL21 and CXCL13, which are essential for normal DLN organization and function. Our data reveal that the mechanism of this suppression is dependent on *S. typhimurium* LPS (sLPS). The decrease in CCL21 expression involves interaction between sLPS and CCL21-producing cells within DLNs, triggering a distinct Toll-like receptor 4 (TLR4)-mediated host signaling response. In this response, suppressor of cytokine signaling-3 (Socs3) is upregulated, which negatively regulates mothers against decapentaplegic homolog-3 (Smad3)-initiated production of CCL21. Disruption of lymph node architecture and cellular trafficking enhances *S. typhimurium* virulence and could represent a mechanism of immune suppression used by pathogens that primarily target lymphoid tissue.

Efficient adaptive immune responses to invading pathogens require secondary lymphoid tissues to monitor the tissue they drain for antigens and to provide an organized environment to amplify the adaptive response. Both the entry of cells into the lymph node and their organization within it are dependent on stable gradients of homeostatic chemokines^{1–3}. Cellular trafficking into lymph nodes occurs by two routes: via the afferent lymph, the origin of antigen-laden dendritic cells (DCs), following a gradient of CCL21, or through specialized blood vessels, called high endothelial venules (HEVs), which promote the entry of lymphocytes in a process also dependent on homeostatic chemokines⁴. After entry through HEVs, T and B cells segregate themselves, forming distinct regions within the lymph node cortex⁴. CXCL13 expression is confined to the B cell follicles², whereas CCL21 and CCL19 are expressed in T cell zones⁵, and this compartmentalization relies on gradients of these two chemokines. The recruitment of the key players of adaptive immunity to the lymph node and their organization within it are vital to produce a timely response to a pathogen challenge, as illustrated by the delayed or deficient adaptive responses in mice lacking either T cell zone- or B cell zone-defining chemokines^{6,7}. In spite of the many

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AUTHOR CONTRIBUTIONS

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essential and varied contributions to lymph node function by homeostatic chemokines, the signals that control and maintain their expression are not adequately understood.

Notwithstanding their central role in initiating adaptive immune responses, lymph nodes are the primary targets of infection by several pathogens^{8–11}. *S. typhimurium* is a versatile host-adaptable enteric pathogen known to traffic through lymphoid tissue including the mesenteric lymph nodes (MLNs) and spleen after crossing the gastrointestinal barrier. The virulence of *Salmonella* has been attributed to its ability to invade and grow within and outside of various host cells, rapidly reaching overwhelming numbers¹¹. Although both T cells and B cells are instrumental in bacterial clearance and host survival^{12–15}, adaptive immune responses are surprisingly ineffective in controlling *S. typhimurium*, and unattenuated strains are lethal to mice. We began this work by investigating the effect of *S. typhimurium*'s preferential invasion of lymph node tissue to the structure and function of this organ.

RESULTS

Salmonella alters draining lymph node architecture

To eliminate the limitations of examining *S. typhimurium* pathogenesis in the highly disseminated lymphoid structures of the gut and to facilitate the inspection of *S. typhimurium*-infected lymph nodes, we used a footpad model of infection where the site is drained by a single draining lymph node (DLN), the popliteal node. For comparison, we examined mice challenged with *Escherichia coli*, a pathogen that does not typically infect secondary lymphoid tissue, and *Listeria monocytogenes*, a virulent Gram-positive bacterium that also can infect DLNs. When we stained *S. typhimurium*-infected DLN sections for lymphocytes, we observed disrupted B and T cell zones (Fig. 1a), whereas lymph nodes from *E. coli*- or *L. monocytogenes*-infected mice were morphologically similar to those of saline controls. This architecture disruption was not due solely to the presence of bacteria in the DLN, as *L. monocytogenes*, unlike *E. coli*, also infected the DLN in comparable numbers (Supplementary Fig. 1a). These observations indicate that the presence of *S. typhimurium* within the DLN uniquely triggers marked changes in its architecture.

Altered trafficking in DLNs is LPS and TLR4 dependent

While inspecting *S. Typhimurium*-infected DLNs, we observed that one mutant strain of *S. typhimurium* did not cause the architectural changes observed with its wild-type (WT) parent strain (Fig. 1b and Supplementary Figs. 2 and 3). This mutant lacks the *msbB* gene, whose product allows modifications to LPS that are essential for recognition by its receptor, TLR4, on host cells¹⁶. TLR activation by bacteria has been well studied in antigen-presenting cells and cells at the host-environment interface, resulting in the paradigm that TLR4 recognition of LPS is instrumental to the initiation of innate immune responses and can influence the magnitude and character of adaptive responses¹⁷. In contrast, the utility of surveillance by TLR4 during *S. typhimurium* infection is uncertain, as the *msbB* mutant is surprisingly attenuated, suggesting that LPS could be a virulence factor of *S. typhimurium*^{18,19}. When we examined lymph node architecture during infection with *msbB*-mutant bacteria, we found that loss of architecture was not evident (Fig. 1b), although comparable numbers infect DLNs (Supplementary Fig. 1b). Thus, the disruption of DLN architecture during *S. typhimurium* infection appears to be mediated by the presence of sLPS.

In light of the inappropriate localization of cells within the lymph node during WT *S. typhimurium* infection, we suspected that cellular trafficking into the lymph node might also be affected. Therefore, we examined cell types crucial to the initiation of adaptive responses

within lymph nodes, DCs, T cells and B cells during infection with WT or *msbB*-mutant *S. typhimurium*. Compared with the *msbB* mutant, WT *S. typhimurium*-infected DLNs had lower T cell numbers (Fig. 1c and Supplementary Fig. 4), although the B cell compartment of the DLN was not substantially altered (Supplementary Fig. 5a,b). However, B cells in *S. typhimurium*-infected nodes frequently localized in clusters around HEVs rather than peripheral B cell zones (Supplementary Fig. 5c). Under both steady-state and inflammatory conditions, DCs localize within the T cell zones of DLNs in response to chemokines CCL21 and CCL19 (ref. 4). Although we did not observe a change in the percentage of lymph node DCs (data not shown), there was a striking change in the localization of these cells within WT *S. typhimurium*-infected DLNs (Fig. 1d). These studies suggest that WT *S. typhimurium*, through expression of sLPS, has the ability to interfere with the organized trafficking of key initiators of adaptive immune responses into and within secondary lymphoid tissue.

To determine the cause of the observed architectural changes, we hypothesized that *S. typhimurium*-mediated disruption of DLNs might involve the dysregulation of homeostatic chemokine expression, as both the cellular organization of the DLN and the trafficking of cells into it are dependent on the local production of these chemokines. We quantified expression of two T cell zone-defining chemokines, CCL21 and CCL19, and two B cell chemoattractants, CXCL13 and CXCL12, within DLNs 24 h after *S. typhimurium* infection and observed lower amounts of both CCL21 and CXCL13 compared with DLNs of saline-injected or *E. coli*-infected mice (Fig. 2a). The levels of CCL19 and CXCL12 remained relatively constant (Fig. 2a and Supplementary Fig. 5d), as did surface expression of CCR7 on T cells and DCs and CXCR5 on B cells (Supplementary Fig. 6), highlighting specificity to the affected pathways. When we compared CCL21 and CXCL13 expression in WT and *msbB* mutant-infected DLNs, only the WT strain lowered CCL21 and CXCL13 expression (Fig. 2b), in spite of comparable numbers of bacteria within DLNs (Supplementary Fig. 1b), supporting a role for LPS in the suppression of homeostatic chemokine production during *S. typhimurium* infection. These trends in chemokine expression were corroborated by western blotting (Supplementary Fig. 7), and staining for these chemokines in tissue sections from WT or *msbB*-mutant *S. typhimurium*-infected DLNs visually supported the decrease in CCL21 and CXCL13 during WT *S. typhimurium* infection at the protein level (Fig. 2c). To determine whether sLPS was sufficient for downregulation within CCL21 producing cells, we used the SVEC4-10 cell line, which retains many functional characteristics of HEVs *in vitro*, making it an appropriate and frequently used model²⁰. Treatment for 24 h with sLPS lowered CCL21 expression, as did treatment with WT *S. typhimurium* (Fig. 2d). In contrast, cells treated with *msbB*-mutant *S. typhimurium* had similar CCL21 expression to untreated cells (Fig. 2d). These results illustrate that, *in vitro*, sLPS is sufficient to recapitulate the reduction of homeostatic chemokines observed within WT *S. typhimurium*-infected DLNs, implying a direct interaction between sLPS and chemokine-producing cells results in the observed suppression of homeostatic chemokine expression.

We next hypothesized that sLPS is sensed by chemokine-producing cells within the lymph node through its well characterized cell surface receptor, TLR4. When we infected TLR4-deficient (TLR4-KO) mice with WT *S. typhimurium*, they maintained normal lymph node architecture (Fig. 3a) and relative levels of CCL21 and CXCL13 expression during *S. typhimurium* infection (Fig. 3b), and, again, these DLNs contained comparable numbers of bacteria (Supplementary Fig. 1c). Little is known about the regulation of homeostatic chemokines during infection, yet it has been reported that interferon- γ (IFN- γ) may act to decrease the abundance of homeostatic chemokines at later time points during infection, potentially as part of host-initiated contraction of the adaptive response²¹. We found that the mechanism of LPS-induced downregulation of homeostatic chemokine expression observed during *S. typhimurium* infection was independent of IFN- γ (Supplementary Fig. 8).

Therefore, LPS seems to initiate a unique signaling program within *S. typhimurium*-infected DLNs to decrease homeostatic chemokine expression.

sLPS-induced CCL21 suppression involves Socs3 and Smad3

To begin a mechanistic investigation of the TLR4-dependent suppression of homeostatic chemokines, we initially considered signaling intermediaries that are known to be modulated downstream of TLR4 stimulation. Socs3 is involved in the negative regulation of many signaling events and is upregulated in response to *S. typhimurium* or sLPS stimulation in some cell types^{22,23}. Intracellular staining for Socs3 showed that increased production occurs within certain cells in DLNs (Supplementary Fig. 9a), and staining for the HEV-specific marker peripheral lymph node addressin (PNAd) revealed colocalization (Fig. 4a). This induction of Socs3 did not occur in mice infected with the *msbB*-mutant strain (Supplementary Fig. 9b). We next focused on the *S. typhimurium*-induced decrease in CCL21, as this chemokine has a well characterized source within lymph nodes, the HEVs, and an appropriate cell line to facilitate molecular studies, SVEC4-10, which we had observed to be responsive to sLPS (Fig. 2d). Treatment of SVEC4-10 cells with WT *S. typhimurium* or sLPS, but not *msbB*-mutant *S. typhimurium*, was sufficient to induce upregulation of Socs3 (Fig. 4b). To investigate the possibility that sLPS-induced Socs3 production in HEVs suppresses the expression of CCL21, we prevented Socs3 induction in response to sLPS with siRNA, after which sLPS stimulation failed to lower CCL21 expression (Fig. 4c). Overexpression of Socs3 in SVEC4-10 cells was also sufficient to lower CCL21 expression compared to control cells (Fig. 4d). These data suggest that Socs3 is upregulated in response to *S. typhimurium* or sLPS and acts upstream of CCL21 as a negative regulator.

To identify a transcription factor contributing to suppression of CCL21 expression during *S. typhimurium* infection, we focused on candidates known to respond to LPS or *S. typhimurium*. One such factor was Smad3, as Socs3 has been shown to bind phosphorylated Smad3 (P-Smad3) in LPS-activated macrophages, preventing the nuclear translocation of P-Smad3 (ref. 24). In the event that Smad3 signaling was similarly affected in our system, we expected LPS-induced upregulation of Socs3 to sequester Smad3 in the cytosol and destabilize any downstream pathways. Our data suggest that there is a basal level of nuclear translocation by Smad3 within untreated SVEC4-10 cells, which can be abrogated by treatment with WT *S. typhimurium* or sLPS (Fig. 4e). Treatment with *msbB*-mutant *S. typhimurium* had no apparent effect on the amount of nuclear Smad3 (Fig. 4e). Cytoplasmic fractions, in contrast, showed consistent levels of Smad3 in all treatments (Fig. 4e). These data support the hypothesis that sLPS-induced signaling is capable of modulating Smad3 nuclear translocation in SVEC4-10 cells; therefore, we next investigated whether Smad3 signaling acts upstream of CCL21 production. We treated SVEC4-10 cells with siRNA against Smad3 and observed a significant corresponding decrease in CCL21 expression (Fig. 4f). Additionally, overexpression of Smad3 in SVEC4-10 cells increased CCL21 messenger RNA levels (Fig. 4g). Therefore, normal CCL21 production by HEVs may be dependent on a basal level of Smad3 signaling.

LPS is a virulence factor of *S. typhimurium* in vivo

To examine the role of LPS in *S. typhimurium* pathogenesis, we compared survival of mice infected by WT *S. typhimurium*, capable of suppressing homeostatic chemokine expression, to the *msbB* mutant, where the suppression was no longer active. Oral challenge of mice with 1×10^4 WT *S. typhimurium* resulted in 100% mortality, whereas an equivalent inoculation with *msbB*-mutant *S. typhimurium* caused only 20% mortality (Fig. 5a). In TLR4-KO mice, WT and *msbB*-mutant *S. typhimurium* showed similar virulence, illustrating that the difference in virulence between these two strains in our model is likely to

be dependent on host TLR4 recognition rather than an intrinsic defect of *msbB*-mutant *S. typhimurium* (Supplementary Fig. 10). Additionally, there were higher bacterial numbers in the spleen and liver of WT *S. typhimurium*-infected mice (Fig. 5b), pointing to a reduced ability of WT *S. typhimurium*-infected hosts to contain infection. Collectively, these data emphasize the increased virulence of WT *S. typhimurium*, able to activate host TLR4 signaling and suppress homeostatic chemokine expression, over the *msbB*-mutant strain that cannot.

To assess the impact of sLPS-mediated suppression of homeostatic chemokines on *S. typhimurium* virulence, we examined the pathogenesis of *msbB*-mutant *S. typhimurium* during the blockade of CCL21 and CXCL13 by neutralizing antibodies. We expected that by artificially disrupting the architecture of lymphoid tissue during infection with *msbB*-mutant *S. typhimurium*, which would be unable to trigger the LPS-TLR4-dependent disruption, the role of adaptive responses in countering *S. typhimurium* pathogenesis would become apparent. We examined bacterial numbers in the liver and spleen at 4 d to allow enough time for adaptive responses to be initiated while limiting the window during which chemokine levels must be suppressed (an experimental timeline is provided in Supplementary Fig. 11a). Combined blocking of chemokines CCL21 and CXCL13 induced observable changes in the DLN architecture by 12 h (Supplementary Fig. 11b), resembling WT *S. typhimurium* infection (Fig. 1), which persisted throughout the experimental protocol (Supplementary Fig. 11c). As expected, given the known function of homeostatic chemokines in promoting the efficiency of adaptive responses, these mice showed reduced T cell activation in DLNs (Supplementary Fig. 11d). Additionally, higher bacterial numbers were present in the spleen and liver of mice undergoing chemokine blockade treatment compared to control-treated mice (Fig. 5c). In contrast, administration of neutralizing antibodies against either CCL21 or CXCL13 individually resulted in lower bacterial numbers in these organs than in mice given the combined antibody treatment against both CCL21 and CXCL13 (Fig. 5c and Supplementary Table 1). Furthermore, simultaneous blockade during the initial stages of infection significantly increased the mortality of mice infected with *msbB*-mutant *S. typhimurium* subsequent to the neutralizing antibody protocol (Fig. 5d). These data point to an essential contribution by homeostatic chemokines to ultimately limiting *Salmonella* numbers *in vivo* and promoting host survival.

Presumably, reduced concentrations of homeostatic chemokines and disrupted lymph node architecture adversely affect the host's ability to contain and clear *S. typhimurium* infection through the functions of the adaptive immune system, an assumption supported by the known role of homeostatic chemokines in promoting adaptive responses and data in our model showing impaired T cell activation (Supplementary Fig. 11d). To test this, we undertook studies to assess the contribution of the adaptive response to limiting *msbB*-mutant *S. typhimurium* virulence, as infected mice maintain normal levels of homeostatic chemokines and can survive a dose equivalent to a lethal dose of WT *S. typhimurium*. Comparing the pathogenesis of *msbB*-mutant *S. typhimurium* in athymic nude mice to heterozygous controls, we found that control mice largely survived challenge with the *msbB*-mutant strain, whereas nude mice had 100% mortality (Fig. 5e). These data support existing reports that adaptive responses, in this case T cell function, are potentially indispensable in host defense against *S. typhimurium* and demonstrate that the reduced virulence observed during infection by *S. typhimurium* lacking functional LPS requires T cells. Nude mice infected with the *msbB*-mutant strain showed mortality similar to WT mice infected with WT *S. typhimurium* (Fig. 5f), suggesting that the host's adaptive response affords a degree of protection against *S. typhimurium* in the absence of sLPS-mediated immunosuppression and that the reduced virulence of this strain can at least partially be accounted for by the function of the adaptive immune system. Cumulatively, our data reveal a mechanism to explain why adaptive responses against *S. typhimurium* are largely

ineffective and suggest that the sLPS-mediated disruption of the lymph node and its ramifications for adaptive immunity constitute a major virulence factor for *S. typhimurium*.

DISCUSSION

It has long been observed that a striking aspect of *Salmonella* pathogenesis is its targeting of lymphoid tissue for dispersal, a virulence strategy that can be extended to few other major bacterial pathogens. The cellular components of lymph nodes are responsible for mounting adaptive immune responses, but lymph nodes functionally exist to facilitate this response and create an environment of efficient collaboration for those constituents. Organization of the lymph node relies on the function of homeostatic chemokines such as CCL21 and CXCL13, amounts of which we observe are reduced during WT *S. typhimurium* infection. Although neither homeostatic chemokines nor organized lymphoid tissue are required to observe adaptive immune responses, these factors greatly increase their efficiency. In the case of infections with highly virulent and invasive pathogens, inadequate or delayed responses can affect host survival. Indeed, we observed that the reduced homeostatic chemokine expression, architecture disruption and host mortality that occur as a result of WT *S. typhimurium* infection are abrogated when the bacteria do not express LPS that is recognizable by TLR4. The unique role of LPS as a virulence factor for *S. typhimurium* may rely on the delivery of relatively high concentrations of LPS to the DLN as a result of *S. typhimurium* invasion and replication within lymphoid tissue (Fig. 6a). Perhaps for this reason, *E. coli*, which is also a Gram-negative pathogen but is predominately opportunistic, with limited abilities to reach lymph nodes, did not initiate similar architectural changes in our studies (Fig. 1a), and, in support of this, we have observed that direct treatment of SVEC4-10 cells with *E. coli* *in vitro* can cause a reduction of CCL21 production similar to that caused by *S. typhimurium* (Supplementary Fig. 12).

The highly evolutionarily conserved Toll family of proteins forms the cornerstone of innate defense against microbial pathogens for species ranging from fruit flies to humans and mice. The relevance of quick pathogen recognition by Toll-like receptors at mucosal surfaces and other sites of pathogen entry is well recognized, yet the relevance of TLR signaling on cells located in deep tissue is less clear. This study reveals that LPS stimulation of TLR4 may not be advantageous to the host when occurring within lymph nodes. The dependence of the disruption of DLN architecture on TLR4 signaling during *S. typhimurium* invasion to the DLN highlights the possibilities of unique signaling pathways occurring when cells specialized for functions other than pathogen surveillance are stimulated through TLR4. This may be the case within the CCL21-producing HEVs, where the presence of LPS and signaling through TLR4 initiate the upregulation of Socs3. This, in turn, seems to negatively regulate the transcription factor Smad3 and reduce CCL21 expression (Fig. 6b). Although these signaling intermediaries are known to be TLR4 responsive, their mechanistic involvement in the regulation of CCL21 reveals the likelihood that the pathways downstream of TLR4 can have unexpected effects when stimulated in highly specialized cell types with unique signaling circuitry. Notably, we have observed that Socs3 is also upregulated in cells expressing markers for follicular dendritic cells (Supplementary Fig. 13), which are known to produce CXCL13 (ref. 5), suggesting that the suppression of CXCL13 could involve a similar signaling mechanism.

In the long evolutionary interplay between mammals and virulent pathogens, hosts have been forced to develop a complex arsenal of immune defenses against pathogens that, in turn, continue to expand mechanisms of subversion or co-optation. *S. typhimurium* is one such host-adapted pathogen, and the targeting of lymphoid tissue for disruption through host TLR4 signaling represents a previously unrecognized route by which this species exploits host defenses and prevents eventual targeting by the adaptive immune system.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

S. typhimurium alters cellular trafficking within DLNs. (a,b) Tissue sections from popliteal DLNs, stained for B cells (B220, green) and T cells (CD3, blue) 24 h after subcutaneous footpad injection of saline or 1×10^5 colony-forming units (CFU) of *S. typhimurium* SL1344, *E. coli* or *L. monocytogenes* (a) or WT or $\Delta msbB$ *S. typhimurium* (b). (c) The percentage (left) and total number (right) of T cells in *S. typhimurium*-infected popliteal lymph nodes, 24 h after subcutaneous footpad injection of 1×10^5 CFU of WT or $\Delta msbB$ *S. typhimurium*. * $P < 0.01$; ** $P < 0.05$. Error bars represent means $\pm$ s.e.m., and data are representative of two similar independent experiments; $n = 3$ per experiment. (d) DLN tissue sections infected with WT or $\Delta msbB$ *S. typhimurium* and stained for B220, CD3 and CD11c (red). Merged images are the left panels; isolated channel to the right shows CD11c⁺ cells. Scale bars, 200 μ m.

Figure 2.

LPS induces the specific downregulation CCL21 and CXCL13 during *S. typhimurium* infection. (a) mRNA levels of homeostatic chemokines CCL21, CCL19 and CXCL13 in DLNs 24 h after footpad infection with *E. coli* or WT *S. typhimurium*. (b) The relative expression of CCL21 and CXCL13 in DLNs 24 h after footpad infection with WT or $\Delta msbB$ *S. typhimurium*. (c) DLN tissue sections stained for a HEV marker, PNAd, and CCL21 within T cell zones (left panels) or CXCL13 within B cell zones (right panels), 24 h after footpad infection. Scale bar = 200 μ m. (d) Levels of CCL21 expression in SVEC4-10 cells treated with WT or $\Delta msbB$ *S. typhimurium* or with sLPS. For all panels a–b,d, * P < 0.05; error bars represent means $\pm$ s.e.m. All panels are representative of two or more independent experiments for a total $n = 3$.

Figure 3.

Homeostatic chemokine suppression is dependent on TLR4 expression. **(a)** Tissue sections from TLR4-KO mice before and 24 h after bacterial instillation, stained to reveal DLN architecture. (B cell zones: B220, green; T cell zones: CD3, blue.) Scale bar, 200 μ m. **(b)** Relative mRNA levels in WT or TLR4-KO mice infected with *S. typhimurium* versus saline-injected controls. * $P < 0.05$; error bars represent means $\pm$ s.e.m. Similar data were acquired in three independent trials.

Figure 4.

Socs3 and Smad3 modulate LPS-mediated CCL21 suppression. **(a)** DLN sections, 24 h after footpad injection of 1×10^5 CFU of *S. typhimurium*, stained for the HEV marker PNAd and Socs3. The bottom panels show co-localization of these markers by multi-channel image (left) and software-generated co-localization image (right). Scale bar, 25 nm. **(b)** Expression of Socs3 in SVEC4-10 cells 6 h after treatment with WT or $\Delta msbB$ *S. typhimurium* or LPS. $*P < 0.01$. **(c)** Socs3 expression 6 h after treatment with (+) or without (–) sLPS (left) or CCL21 expression 24 h after treatment (right) in SVEC4-10 cells treated with siRNA. For left graph, $*P < 0.001$. For right graph, $*P < 0.01$. **(d)** Expression of Socs3 (left) and CCL21 (right) in Socs3-overexpressing (Socs3-O) cells compared to control cells. $*P = 0.02$. **(e)** Western blots, probed using an antibody against P-Smad3, after SDS-PAGE separation of proteins from SVEC4-10 cells treated with WT or $\Delta msbB$ *S. typhimurium* or sLPS. Blots contain either cytoplasmic fractions or proteins pulled down from nuclear fractions using an antibody against Smad3. **(f)** Relative Smad3 and CCL21 expression, determined by real-time PCR, after Smad3 knockdown (KD) in SVEC4-10 cells using siRNA. $*P = 0.002$. **(g)** Relative levels of Smad3 and CCL21 in SVEC4-10 cells overexpressing Smad3 (Smad-O). For Smad3 expression, $*P = 0.03$. For CCL21 expression, $*P = 0.04$. For all panels, error bars represent means $\pm$ s.e.m., and data are representative of three or more trials.

Figure 5.

Adaptive immune contributions to limiting *S. typhimurium* infection. (a) Survival of mice inoculated orally with 1×10^4 CFU of WT *S. typhimurium* compared to mice challenged with the $\Delta msbB$ strain. (b) Quantification of bacterial numbers in the liver and spleen after 4 d of infection by WT or $\Delta msbB$ *S. typhimurium*. (c) Quantification of bacterial numbers in the spleen and liver in mice infected with $\Delta msbB$ *S. typhimurium* that had undergone combined or individual chemokine blockade or control antibody (Ig) administration (see Online Methods). $P < 0.03$, by one-way analysis of variance. See Supplementary Table 1 for further statistical analysis of panels b and c. For b and c, error bars represent means $\pm$ s.e.m.,

and data are representative of two or more independent experiments, except in the case of individual chemokine blockade, included in one trial for a total $n = 3$. **(d)** Survival curves of mice treated with control or combined chemokine-specific antibody treatment, $P = 0.02$. **(e)** Survival of nude mice on a C57BL/6J background compared to heterozygous control mice after oral infection with the $\Delta msbB$ strain. $P = 0.05$. **(f)** Survival of nude mice after oral infection with the $\Delta msbB$ strain compared to WT mice infected orally with WT *S. typhimurium*. $P = 0.47$.

Figure 6.

Model of *S. typhimurium*-induced suppression of homeostatic chemokines. **(a)** Schematic illustrating the proposed mechanism of *S. typhimurium*-induced lymph node architecture changes. During lymph node homeostasis, the expression of chemokines within the DLN contributes to the maintenance of normal architecture. CCL21 and CXCL13 are two key chemokines that promote the formation of T and B cell zones, respectively. Subsequent to the initial invasion of *S. typhimurium* into the host peripheral tissues, *S. typhimurium* invades further into the host, reaching the DLN. Once in the DLN, *S. typhimurium*'s presence disrupts cellular trafficking within the DLN, characterized by disorganized B and T cell zones and altered DLN cellular constituents. The disruption of *S. typhimurium*-infected

DLNs is accompanied by a reduced expression of CCL21 and CXCL13. Our data suggests that the mechanism of CCL21 reduction within HEVs is dependent on LPS signaling through TLR4. **(b)** In the depicted model of TLR4 signaling, engagement of TLR4 results in upregulation of Socs3, a negative regulator of the transcription factor, Smad3. At basal levels, phosphorylated Smad3 is able to enter the nucleus, where it functions upstream of CCL21 expression in the SVEC4-10 model of HEVs. The upregulation of Socs3 in response to TLR4 signaling results in sequestration of Smad3 in the cytoplasm and reduces Smad3 promoted CCL21 expression.