A Mouse Model of Shigellosis by Intraperitoneal Infection

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In human and nonhuman primates, Shigella spp. cause bacillary dysentery by invading colon epithelium and promoting a strong inflammatory response; however, adult mice are resistant to oral Shigella infection. In this study, intraperitoneal challenge with virulent S. flexneri 2a (YSH6000) resulted in diarrhea and severe body weight loss in adult B6 mice. Of note, virulent S. flexneri 2a could invade and colonize not only systemic tissues but also the serosa and lamina propria region of the large intestine. In addition, epithelial shedding, barrier integrity, and goblet cell hyperplasia were found in the large intestine by 24 hours post-intraperitoneal Shigella infection. Of note, predominant expression of proinflammatory cytokines and chemokines were found in the large intestine after intraperitoneal challenge. Monocytes played a critical role in attenuating diarrhea and in providing protective efficacy against intraperitoneal Shigella infection. Most importantly, mice prevaccinated with attenuated S. flexneri 2a (SC602) strain were protected against intraperitoneal challenge with YSH6000. When taken together, these findings show that intraperitoneal challenge with virulent S. flexneri 2a can provoke bacillary dysentery and severe pathogenesis in adult mice. This model may be helpful for understanding the induction mechanism of bacillary dysentery and for evaluating Shigella vaccine candidates.

Keywords. intraperitoneal route; S. flexneri; shigellosis; CCR2+ monocytes; mouse model.

INTRODUCTION

Gram-negative Shigella spp. are well-known contagious intracellular pathogens that can invade hosts via spoiled food or contaminated water [1, 2]. The organisms penetrate several virulent proteins through a type III secretion system (T3SS) that disturbs the host defense system and facilitates infection [3, 4]. Shigella organisms endocytosed by M cells are entrapped by the membrane vacuoles of resident macrophages or epithelial cells, but they can rupture the vacuole membranes and disseminate into the epithelial cell cytoplasm, where they multiply and spread within as well as into adjacent epithelial cells [5, 6]. Oral Shigella infection causes bacillary dysentery by promoting inflammatory responses that are accompanied by fever, abdominal cramps, and tenesmus in human and nonhuman primates [7, 8].

Although mortality caused by shigellosis is declining in most age groups, one exception is children <5 years old because of continuing unsanitary conditions in many areas of the world [2, 9]. To develop a safe and effective vaccine, it is essential to understand the pathogenesis of shigellosis in diverse animal models that replicate human symptoms. Both our current understanding of the pathogenesis of bacillary dysentery and our ability to evaluate vaccine candidates are based largely on studies performed in in vitro cell lines [4, 10] and in vivo infection models such as guinea pig keratoconjunctivitis and rabbit ligated ileal loops [11, 12]. Of the nonhuman primates considered for infection models, mice are readily available, inexpensive, and easy to handle. However, we found no reports about the relationship of shigellosis and intestinal pathology and bacillary dysentery in adult or aged mice. Such a model would be more useful for assessing immune responses and protective efficacy. Toward that end, we

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used an intraperitoneal Shigella infection route in adult mice. We found that intraperitoneal challenge with virulent S. flexneri provoked severe diarrhea and acute inflammation in both systemic and mucosal tissues and mimicked human bacillary dysentery. Mice prevaccinated with attenuated vaccine S. flexneri 2a (SC602) strain had effective protection against intraperitoneal challenge with virulent S. flexneri 2a (YSH6000). Our results suggest that Shigella challenge through the peritoneal cavity can be used for testing the immunogenicity and protective efficacy of vaccine candidates for preclinical assessment.

**MATERIALS AND METHODS**

**Mice**

Balb/c and C57BL/6 (B6) mice were purchased from Charles River Laboratories (Orient Bio Inc, Sungnam, Korea). Ccr2−/− mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were maintained under specific pathogen-free conditions in the experimental facility at the International Vaccine Institute (Seoul, Korea) where they received sterilized food and water ad libitum. All animal experiments described above were approved by Institutional Animal Care and Use committees.

**Bacterial Strain and Infection**

Bacteria were grown in trypticase soy broth (TSB) at 37°C overnight and reincubated with 1% precultured bacteria in fresh media (up to O.D. 0.8–0.9). For bacterial infection, groups of B6 mice were administered bacteria (5 × 10⁸ colony-forming units [CFU]) through intraperitoneal, oral, and intravenous routes. For vaccination studies, mice were intranasally administered SC602 (5 × 10⁸ CFU). The actual bacterial dose given was confirmed by plating serial dilutions onto TSB agar plates.

**Fecal Pathology**

Pathologic scoring of feces was performed as described elsewhere [13]. In brief, fresh feces were categorized by the following parameters: consistency (0–3; normal < loose < soft < hard), color (0–3; brown < yellow < light green < blue-green), and cumulative numbers of diarrhea episodes (0–3).

**Confocal Analysis**

For MUC2 staining, colon tissue was cut longitudinally and dropped into Canoy solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) in 4% formaldehyde for 20 minutes [14]. Then frozen sections were sequentially reacted with rabbit anti-Muc2 immunoglobulin G (IgG) Ab (Santa Cruz Biotechnology, Santa Cruz, CA), biotinylated goat anti-rabbit IgG (GeneTex, San Antonio, TX), and Alexa Flou streptavidin-488 (Molecular Probes, Eugene, OR) at room temperature for 2 hours and viewed under a confocal scanning laser microscope (Zeiss).

**Pathologic Score**

Pathological scores were based on the following parameters: infiltration of inflammatory cells (0–4), epithelial shedding (0–4), loss of barrier integrity (0–4), and goblet cell hyperplasia (0–4).

**Cytokine and Chemokine Levels in the Intestine Tissue**

Cytokine and chemokine levels in the supernatant were measured using FlowCytomix kit (eBioscience, San Diego, CA) according to the manufacturers’ instructions.

**Flow Cytometry Analysis**

Cells were collected from large intestine and stained with MHC class II eFluor710 (AF6–120.1, eBioscience), CD11b FITC (M1/70, BD Biosciences), Ly6C APC (AL-21, BD Biosciences), and Gr-1 PE (RB6–8C5, eBioscience). Flow cytometry data collection was performed by LSR II (BD Biosciences) or Calibur flow cytometers (BD Biosciences) and files were analyzed using FlowJo software (Tree Star, Ashland, OR).

**RESULTS**

**Intraperitoneal Shigella Infection Can Provoke Diverse Pathological Symptoms**

As an alternative infection route for shigellosis in adult mice, 6-week-old B6 mice were infected intraperitoneally with virulent S. flexneri 2a (YSH6000). In order to determine optimal doses for intraperitoneal infection, groups of mice were injected with 5 × 10⁷, 5 × 10⁸, or 5 × 10⁹ CFU of S. flexneri 2a strain (Supplementary Figure 1A). At 5 × 10⁸ CFU, mice had significant weight loss by 3 days after infection, and all died by day 7. Thus, we used this dose for subsequent experiments. Of note, both B6 and BALB/c mice had severe diarrhea by 2 hours (Figure 1A; left) and the symptoms peaked 6 hours and continued until 12 hours as scored by fecal pathology (color and consistency) after intraperitoneal S. flexneri 2a infection (Figure 1A, right). Mice challenged intraperitoneally with S. flexneri 2a showed body weight loss until day 3 when they began to die of sepsis (Figure 1B). In contrast, intravenous infection with S. flexneri 2a (5 × 10⁸ CFU) did not produce fecal pathology although the mice died within 24 hours. Colon length, which is one marker for evaluating colon pathology, shortens in a time-dependent manner after intraperitoneal infection (Figure 1C).

To exclude the possibility that diarrhea might be a symptom of a host defense mechanism not specific for Shigella strains, groups of mice were infected intraperitoneal with virulent Salmonella typhimurium [UK-1 strain, 1 × 10⁷ CFU (LD₅₀)], but no diarrhea resulted (data not shown). Collectively, these
data indicate that intraperitoneal challenge with a virulent Shigella strain provokes diarrhea in adult mice that mimics human shigellosis.

**Shigella Organisms Invade and Colonize the Large Intestine of Adult Mice via the Intraperitoneal Route**

To identify whether Shigella organisms can invade and colonize the host through the intraperitoneal route, we determined numbers of bacteria colonies in mucosal and systemic compartments at different postinfection time points. We found significant numbers of Shigella organisms in peritoneal wash samples, the large intestine, and feces from 1 to 24 hours after infection (Figure 2A). Then bacterial colonies declined, but were still detectable at 72 hours (data not shown). Similar patterns of colonization were shown in systemic (ie, blood, spleen, and liver) and other mucosal (ie, small intestine and mesenteric...
Figure 2. Large intestines of adult mice were analyzed for invasion and colonization after intraperitoneal (i.p.) infection with *Shigella flexneri* 2a YSH6000. A, Colony-forming units (CFU) in homogenates of peritoneal wash, large intestines, and feces at each time point after i.p. infection. B, Localization of *Shigella* organisms in large intestine after i.p. infection determined with green fluorescent protein (GFP)-expressing YSH6000. Confocal microscopy was employed to visualize differential depths (z) of tissues (left and middle; scale bar = 50 and 10 µm, respectively.) Right: Bacteria in cells stained with nucleic acid. Scale bar = 5 µm. C, Twenty-four hours after challenge by i.p. and intravenous (i.v.) routes, bacteria were localized in whole large intestines and analyzed by confocal microscopy. Photos were taken by z-stack and then integrated into 2-D images. Scale bar = 20 µm. Data are mean of 3 independent experiments (n = 5 mice per each group). *P < .05, **P < .01, and ***P < .001. Abbreviation: ns, not significant (2-tailed paired t test).
lymphocytic peritoneum (Supplementary Figure 1B). We next used confocal microscopy to determine whether bacteria can invade the large intestine and not simply attach/adhere. We found GFP-expressing *Shigella* spp. in connective tissue of peritoneum and serosa 1 hour following intraperitoneal infection and also in the muscle layer and lamina propria at 24 hours (Figure 2B, left). In addition, z-stack analysis revealed that *Shigella* spp. could invade, reside, and replicate in the inner tissues (Figure 2B, middle and right). To further clarify invasion patterns after intraperitoneal *Shigella* administration, we compared findings in the large intestines of mice infected intraperitoneally and by intravenous injection (Figure 2C). While GFP-expressing *Shigella* spp. given by intravenous injection were trapped in muscle layer blood vessels, abundant bacteria were dispersed and localized in the outer muscle layer and the inner lumen regions of the large intestine at 24 hours after intraperitoneal infection (Figure 2C). These results imply that bacterial invasion into the large intestine via the peritoneal cavity occurs by specific migration via the serosa and muscle layer and not by circulation.

**The Type III Secretion System (T3SS) is Essential for Induction of Diarrhea**

Because *Shigella* organisms manipulate T3SS activity to facilitate infection and colonization in the colonic epithelium [15], we assessed the role of T3SS in the murine shigellosis model by intraperitoneal challenge with T3SS-intact *S. flexneri 2a* (S325) strain. There were no significant pathological changes (ie, diarrhea, body weight loss, and survival rate) in mice post-intraperitoneal challenge with S325 strain (Supplementary Figure 2A and 2B). As expected, we found significantly diminished numbers of S325 strain in the systemic (ie, spleen) and mucosal (ie, small and large intestines and feces) compartments when compared with those of mice given the T3SS-intact YSH6000 strain (Supplementary Figure 2C). Taken together, these results indicate that intraperitoneal challenge with *Shigella* organisms provoke bacillary dysentery in adult mice in a T3SS-dependent manner.

**Severe Tissue Destruction, Inflammation, and Mucin Secretion in the Large Intestine at an Early Time Point After Intraperitoneal Infection**

To further investigate whether intraperitoneal *Shigella* challenge can provoke inflammatory symptoms in the large intestine, we first assessed morphological changes at 3, 24, and 72 hours after infection. Of note, epithelial shedding, cell death in the crypt and villi, and infiltration of inflammatory cells were found at 24 hours, whereas mild symptoms were detected beginning 3 hours after intraperitoneal infection (Figure 3A). Pathological score, including host cell death, epithelial shedding, barrier integrity, and goblet cell hyperplasia, peaked at 24 hours after infection and finally recovered at about 72 hours (Figure 3B). Further, excess mucin secretion by goblet cell hyperplasia (Figure 3C) and increased MUC2 in both protein and messenger RNA (mRNA) levels (Figure 3D and 3E) were found in the large intestine at 24 hours and recovered to normal levels about 72 hours post *Shigella* intraperitoneal infection. However, these pathological changes in the large intestine recovered to near normal levels by 72 hours after infection (Figure 3C-E). The fact that there were higher levels of FITC-dextran in serum of infected mice than in noninfected mice (Figure 3F), indicates permeability of the epithelial cells of the large intestine was significantly increased by intraperitoneal challenge. Taken together, these results demonstrate that diverse inflammation symptoms in the large intestine were provoked by intraperitoneal challenge with *Shigella* spp. that might be the direct cause of the diarrhea.

**Comparison of Gene Expression Levels in the Colon After Oral or Intraperitoneal Challenge With *S. flexneri 2a* Strain**

To further characterize transcription factors involved in the onset of bacillary dysentery by intraperitoneal challenge, we performed genome-wide cDNA microarray analysis of gene-expression profiles of whole colon tissues at the following times and conditions: (1) nil, (2) 3 hours post oral infection, (3) 3 hours post-intraperitoneal infection, and (4) 24 hours post-intraperitoneal infection. We did pair-wise comparison of the differentially expressed genes between *Shigella* infection and nil groups (red spot; >2-fold change and q < 0.05). As expected, dynamic changes of gene expressions in the large intestine by 3 and 24 hours post-intraperitoneal challenge were confirmed when compared with nil and oral infection groups (Figure 4A). Predominant numbers of genes related to the biological process (immunity and defense, apoptosis, transport, cell proliferation, and cell adhesion) were enhanced in the tissue homogenates at 3 and 24 hours post-intraperitoneal infection compared with those in nil and oral infection groups (Figure 4B). We further selected some genes and confirmed mRNA expression by real-time polymerase chain reaction (RT-PCR; Figure 4C). We found mRNA expression levels of antimicrobial activity (eg, *Lcn2*, *S100a8*) [16, 17] and mononuclear cell migration (eg, *Ccl4*, *Cxc11*) were significantly increased by intraperitoneal *Shigella* infection (Figure 4B and 4C). Interestingly, the transcription levels of amphiregulin (*Areg*), which promotes intestinal epithelial regeneration in the context of acute epithelial injury [18], and selectin-P (*Selp*), which mediates the early infiltration of leukocytes into inflamed tissue [19], peaked 3 hours after infection and then significantly decreased until 24 hours (Figure 4C). Taken together, these results suggest that the gene expression levels that are related to intrinsic host defense against bacterial infection significantly increase in the large intestine when *Shigella* organisms invade via the peritoneal cavity.
Figure 3. Pathophysiological changes in large intestines of adult mice after intraperitoneal (i.p.) infection with *Shigella flexneri* 2a YSH6000. A, Hematoxylin-eosin (H&E) staining of large intestine 0, 3, 24, and 72 hours after intraperitoneal (i.p.) challenge with YSH6000 strain. Scale bar = 100 µm. B, Histological score assessment by blind test (n > 5) of infiltration of inflammatory cells (a), epithelial shedding and loss of barrier integrity (b), and goblet cell.
Intraperitoneal Challenge With Shigella Organisms Lead to High Levels of Proinflammatory Cytokines and Chemokines in the Colon

In order to further address whether intraperitoneal challenge with Shigella spp. elicits inflammatory responses in the colon, we determined secretion levels of cytokines and chemokines in the supernatant of colon tissue homogenates at 3 and 24 hours post-intraperitoneal infection. We found that proinflammatory cytokines such as interleukin 1α/β (IL-1α/β), interferon α (IFN-α), interferon γ (IFN-γ), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α) were significantly higher by 3 hours post-intraperitoneal challenge than those of the nil group (Figure 5A). Although high levels of IFN-γ and TNF-α were maintained at 24 hours after intraperitoneal challenge, IL-1α/β, IFN-α, and IL-6 levels peaked at 3 hours and then declined (Figure 5A). In addition, secretion of such chemokines as CCL2, CCL3, CCL4, CCL5, CCL7, and CXCL10, which are well known to be involved in recruiting mononuclear cells, were drastically increased in the colon homogenates post-intraperitoneal infection (Figure 5B). In addition, high levels of IL-6, IFN-γ, and TNF-α were also detected in the serum after intraperitoneal infection (data not shown). Collectively, these data indicate that resident colon Shigella organisms can provoke predominant chemokines, which contribute to recruitment of mononuclear cells into the colon at an early time point. In turn, these may be involved in secretion of proinflammatory cytokines.

Recruitment of CD11b<sup>int</sup>Ly6C<sup>+</sup> Monocytes in the Colon Diminishes Acute Diarrhea Post-intraperitoneal Shigella Challenge

Because major chemokines for recruitment of monocytes were drastically enhanced in the colon post-intraperitoneal challenge with Shigella organisms, we next identified the phenotypes of the recruiting cells and their role in disease onset. Of interest, more CD11b<sup>int</sup>Ly6C<sup>+</sup>Ly6G<sup>−</sup> monocytes had infiltrated the colon 24 hours after intraperitoneal challenge with YSH6000 strain than found in the nil group (Figure 6A). Both proportions and absolute numbers of CD11b<sup>int</sup>Ly6C<sup>+</sup>Ly6G<sup>−</sup> cells (ie, monocytes) were significantly increased in the colon of YSH6000-infected mice (Figure 6B). To determine the exact role of those cell subsets on the onset of diarrhea, we adopted Ccr2<sup>−/−</sup> mice because they have diminished numbers of monocytes. We detected severe diarrhea symptoms in Ccr2<sup>−/−</sup> mice from 2 hours after infection and diarrhea continued until 48 hours post-intraperitoneal infection (Figure 6C). In addition, Ccr2<sup>−/−</sup> mice died much earlier than the wild-type B6 mice (Figure 6D). Morphological changes and clinical colon scores of Ccr2<sup>−/−</sup> mice were greater than those of B6 mice at 3 hours after intraperitoneal infection (Figure 6E). Overall, these results demonstrate that CCR2<sup>−/−</sup> monocytes play a critical role in diarrhea control and susceptibility to infection with Shigella spp.

Intranasal Vaccination With Attenuated Vaccine Strain Protects Mice Against intraperitoneal Shigella Infection

To determine if the intraperitoneal shigellosis model would be useful for measuring protective efficacy of vaccine candidates, adult mice were vaccinated twice intranasally with a live attenuated S. flexneri 2a SC602 vaccine strain [20] at 2-week intervals and then intraperitoneally challenged with virulent S. flexneri 2a YSH6000 strain. There were no significant changes in fecal pathology in the prevaccinated group after intraperitoneal challenge with S. flexneri 2a YSH6000, whereas nonvaccinated wild-type (WT) mice showed severe diarrhea at an early post-infection time point (Figure 7A; left). Of interest, prevaccinated mice were 100% protected against intraperitoneal challenge with S. flexneri 2a YSH6000 (Figure 7A; right). Unlike nonvaccinated mice, the prevaccinated WT mice after YSH6000 intraperitoneal challenge had no epithelial cell shedding, hyperplasia of goblet cells, Paneth cell death, and epithelial cell dislocation in the large intestine (Figure 7B). Collectively, these data indicate that our murine intraperitoneal shigellosis model can be used to measure the protective efficacy of vaccine candidates.

DISCUSSION

Our goal was to create a new shigellosis animal model in adult mice by intraperitoneal challenge with a virulent Shigella strain. We found that an intraperitoneal challenge with S. flexneri 2a provoked acute and severe diarrhea that mimicked human shigellosis (bacillary dysentery) with symptoms that included body weight loss, shortened colon length, epithelial cell shedding, excess mucus secretion, and enhanced secretion of inflammatory cytokines and chemokines. Of most importance, our new murine shigellosis model will be useful for monitoring the safety and protective efficacy of vaccine candidates targeting Shigella infection.

In this study, we demonstrated that the intraperitoneal route is a promising entry site for Shigella bacteria in the systemic and mucosal tissues and that it results in shigellosis-like symptoms in adult mice. Although the intraperitoneal route for
Shigella infection was first reported more than 50 years ago [21], no in-depth study has produced findings that can be considered a new experimental shigellosis animal model. The oral cavity is the most natural route for bacterial entry; however, it is not useful in mice because of intrinsic host defense mechanisms such as host cell death and/or epithelial cell shedding in the early stages of infection (Chang et al, forthcoming). In our study, when mice were challenged intraperitoneally with a virulent Shigella strain, infection occurred in both systemic and mucosal compartments (especially the colon) at very early time points, and bacteria were localized in tissues until death (Figure 2 and Supplementary Figure 2). We previously reported...
Brisk levels of proinflammatory cytokines and chemokines in mouse colon after intraperitoneal (i.p.) challenge with Shigella flexneri 2a. Levels of proinflammatory cytokines (A) and chemokines (B) were determined in the supernatant of colon tissue homogenates at 0, 3, and 24 hours after i.p. infection by Flowcytomix kit. Data are mean ± SEM of 3 independent experiments (n = 5 mice per group); 1-way ANOVA with Bonferroni post hoc test: *P < .05, **P < .01, ***P < .001; ns, not significant. Abbreviations: ANOVA, analysis of variance; SEM, standard error of the mean.
that intestinal epithelial M cells in neonate mice and guinea pigs are not the only entry site for *Shigella* spp. [22, 23]. In the current study, *Shigella* organisms in the peritoneal cavity attached and colonized in the serosa and muscle layer of the colon and successfully entered the lamina propria region (Figure 2). In support of this finding, others have reported that

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**Figure 6.** Protective efficacy of CD11b<sup>int</sup>Ly6C<sup>−</sup>Ly6G<sup>−</sup> monocytes in large intestine against intraperitoneal (i.p.) *Shigella* infection. **A,** Flow cytometry of live cells from whole colon of 6-week-old naive and YSH6000-infected mice. CD11b<sup>int</sup> cell populations were gated from live cells. Numbers are percentages of cell populations. **B,** Frequency and absolute numbers of CD11b<sup>int</sup>Ly6C<sup>−</sup>Ly6G<sup>−</sup> cells in colon. Data are mean ± SEM of 3 independent experiments (n = 3 mice per group); *P* < .05; ns, not significant (1-tailed paired t test). **C,** Feces collected from C57BL/6 and *Ccr2<sup>−/−</sup>* mice at each post-i.p. infection time point. Fecal pathology scored on the basis of color and consistency. **D,** Survival rate of *Ccr2<sup>−/−</sup>* mice and significance relative to C57BL/6 mice. **E,** Mouse large intestine 3 hours after i.p. challenge with *S. flexneri* 2a YSH6000 stained with H&E. Representative H&E stained sections (left). Pathological score after H&E staining (right) by blind method as in Figure 3B. Scale bar = 50 µm. Data are mean ± SEM of 2 independent experiments (n = 5 mice per group); 2-way ANOVA with Bonferroni post hoc test; **P** < .01 and ***P** < .001. Abbreviations: ANOVA, analysis of variance; H&E, Hematoxylin-eosin; SEM, standard error of the mean.
serosal LPS exposure affects the permeability of the rat small intestine in vitro, although mucosal LPS fails to do so [24]. It is possible that serosa-mediated transmission might result from a crack in the muscle layer (Supplementary Figure 3). Because we did not detect bacteria in the colon lumen after intravenous Shigella infection (Figure 2C), we speculate that translocation and replication of bacteria in the colon by intraperitoneal challenge might be not derived from bacteria in the blood stream. These results imply that bacteria that enter via the peritoneal cavity escape the intrinsic host defense system during early infection.

In human acute Shigella dysentery, rectal biopsy specimens show infiltration of different cell types (ie, lymphocytes, plasma cells, and neutrophils), distortion of the crypts, excess secretion of mucin, and edema in the muscularis mucosae and submucosa of the colon [8, 25]. In our study, mice infected intraperitoneally with Shigella strains had similar pathological symptoms including excess secretion of mucin in the colon that might originate from goblet cells (Figure 3). Previous studies suggest that mucin in the colon can be subject to direct colonic infection and inflammation [26–28]. For instance, Muc2<sup>−/−</sup> mice exhibited rapid weight loss, high mortality, and greater bacterial burden when infected with Citrobacter rodentium [27]. Thus, it seems likely that successful intraperitoneal bacterial invasion and colonization of the colon can lead to multiple histological changes that are produced by the intrinsic host defense system in response to intraperitoneal infection.

The host defense systems against Shigella invasion are activated by secretion of chemokines and subsequent recruitment of immune cells to the site of infection [29]. Monocytes, which have been identified as the expression levels of Ly6C, CD11b, and CCR2, play a critical role in antimicrobial immune defense and tissue healing [30, 31]. Previous studies revealed that Ly6C<sup>hi</sup> monocytes are rapidly recruited to infected tissues in a CCR2-dependent manner and involve diverse immune activities and differentiation to TNF/iNOS-producing dendritic cells (DCs), macrophages, and inflammatory DCs against bacterial pathogens such as Listeria monocytogenes, Brucella melitensis, and Mycobacterium tuberculosis [32–34]. Consistent with previous reports, in our study, CD11b<sup>int</sup>Ly6C<sup>hi</sup> monocytes rapidly migrated into the large intestine within 24 hours after intraperitoneal infection with S. flexneri (Figure 6). To support the specific recruitment of Ly6C<sup>hi</sup> monocytes, CCR2-mediated chemokines, including MCP-1 (CCL2) and MCP-3 (CCL7), were substantially increased in the colon by intraperitoneal infection (Figure 5B) [35]. In the absence of CCR2 expression, we assessed severe diarrhea and colon pathology (Figure 6D–F). Overall, our results imply the indispensable role of CCR2<sup>+</sup>Ly6C<sup>hi</sup> monocytes in the
colon mucosa for intrinsic host defense against intraperitoneal S. flexneri infection.

Many researchers, including ourselves, have attempted to develop models for bacillary dysentery using mice, guinea pigs, rabbits, and macaques [12, 23, 36, 37]. Of these, mice are the most suitable in terms of cost, ease of handling, and availability of gene-manipulated animals. The mouse pulmonary pneumonia infection model is commonly used to monitor protective efficacy, but a serious concern is the clinical irrelevance of the inflammation site [38, 39]. Our findings [22] and those of Sansonetti et al [9] suggest the utility of the neonatal murine bacillary dysentery model; however, in this model it is difficult to measure protective efficacy because the symptoms are induced within a narrow window of time, that is, 4–7 days after birth. Thus, the most interesting finding from our current study is that our newly developed bacillary dysentery model is applicable for adult mice and successfully produces clinical symptoms that mimic human shigellosis without any antibiotic treatments. Therefore, we suggest that this murine shigellosis model can be used to predict safety and protective efficacy of vaccine candidates targeting Shigella infection.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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