

Loss of the virulence plasmid by *Shigella sonnei* promotes its interactions with CD207 and CD209 receptors

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Abstract

Introduction. *Shigella sonnei*, the cause of bacillary dysentery, belongs to Gram-negative enteropathogenic bacteria. *S. sonnei* contains a 210 kb virulence plasmid that encodes an O-antigen gene cluster of LPSs. However, this virulence plasmid is frequently lost during replication. It is well-documented that after losing the O-antigen and becoming rough strains, the Gram-negative bacteria may express an LPS core on its surface. Previous studies have suggested that by using the LPS core, Gram-negative bacteria can interact with several C-type lectin receptors that are expressed on antigen-presenting cells (APCs).

Hypothesis/Gap Statement. *S. sonnei* by losing the virulence plasmid may hijack APCs via the interactions of LPS-CD209/CD207.

Aim. This study aimed to investigate if the *S. sonnei* rough strain, by losing the virulence plasmid, interacted with APCs that express C-type lectins of human CD207, human CD209a and mouse CD209b.

Methodology. SDS-PAGE silver staining was used to examine the O-antigen expression of *S. sonnei* WT and its rough strain. Invasion assays and inhibition assays were used to examine the ability of *S. sonnei* WT and its rough strain to invade APCs and investigate whether CD209 and CD207 are receptors for phagocytosis of rough *S. sonnei*. Animal assays were used to observe the dissemination of *S. sonnei*.

Results. *S. sonnei* did not express O-antigens after losing the virulence plasmid. The *S. sonnei* rough strain invades with APCs, including human dendritic cells (DCs) and mouse macrophages. CD209 and CD207 are receptors for phagocytosis of rough *S. sonnei*. Expression of the O-antigen reduces the ability of the *S. sonnei* rough strain to be disseminated to mesenteric lymph nodes and spleens.

Conclusion. This work demonstrated that *S. sonnei* rough strains – by losing the virulence plasmid – invaded APCs through interactions with CD209 and CD207 receptors.

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Keywords: antigen presenting cells (APCs); C-type lectins (CD207 and CD209); *Shigella sonnei*.

Abbreviations: APCs, antigen presenting cells; DCs, dendritic cells; LPS, lipopolysaccharides.

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INTRODUCTION

Shigella spp., the cause of bacillary dysentery, belong to Gram-negative invasive enteropathogenic bacteria that can penetrate mucosal surfaces of guts. The ingestion of as few as 100 bacteria is enough to cause bacillary dysentery [1]. Shigellosis represents a significant public health burden in developing countries, with about 160 million cases occurring annually, predominantly in children under the age of 5 years [2]. The *Shigella* genus comprises four species, including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, and *S. flexneri* and *S. sonnei* cause the most infections [3].

The ratio of species dominance is highly dependent on the socio-economic conditions of an area. In developing countries, including those of sub-Saharan Africa and certain countries in Asia, *S. flexneri* is the dominant cause of shigellosis, responsible for over 60% of infections [4]. In developed countries, such as Europe and North America, *S. sonnei* causes around 80% of shigellosis cases [5]. Countries undergoing socio-economic improvements are also experiencing a change in infections from *S. flexneri* to *S. sonnei* [6]. It appears that the frequency of *S. sonnei* isolation directly correlates with per capita gross

domestic product (GDP) [7]. The primary cause of this association is not fully understood. One hypothesis is that exposure to unsanitized drinking water systems in developing countries results in *Plesiomonas shigelloides* infection, and hence in natural immunity against *S. sonnei*. *P. shigelloides* serotype O17 has a lipopolysaccharide O-antigen identical to that of *S. sonnei* [8]. Also, *S. sonnei* has been shown to possess a functional type 6 secretion system (T6SS), which provides a niche-specific competitive advantage for *S. sonnei* over *S. flexneri* [6].

Most Gram-negative enteropathogenic bacteria contain LPSs located in the outer membrane. The LPSs are composed of three covalently linked domains: lipid A, which is embedded in the outer membrane; the oligosaccharide core (Fig. 1); and the O-polysaccharide or O-antigen, which cover the bacterial surface [9]. The O-antigen is one of the essential components for bacterial survival during infection. For example, *Salmonella enterica*, *Francisella tularensis* and *Burkholderia cepacia* utilize the O-antigen to avoid phagocytosis and to resist lytic action of the complement system [10–12]. Unlike *S. flexneri*, the genes for O-antigen in *S. sonnei* are located on the large 210 kb virulence plasmid

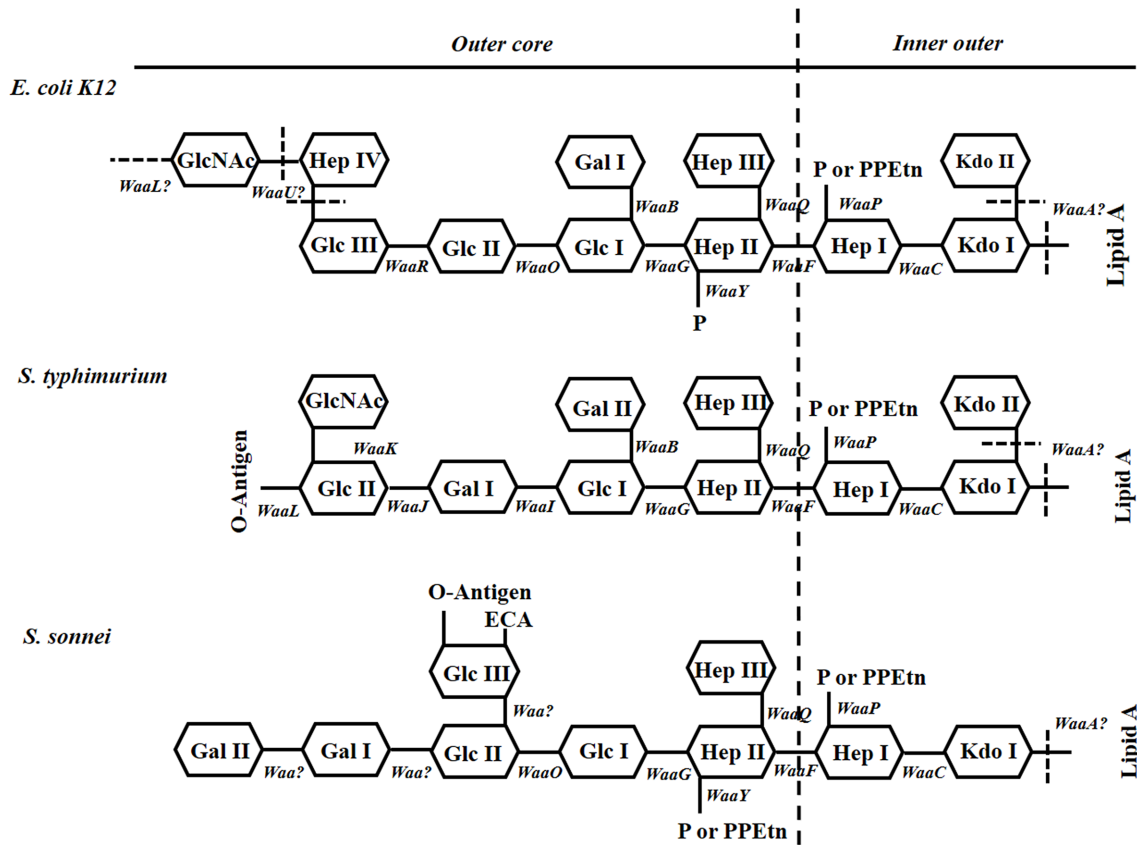


Fig. 1. Structures of inner- and outer-core regions of the LPS or LOS of *E. coli* K12, *S. typhimurium* and *S. sonnei* and the genes involved in their synthesis. Genes involved in the biosynthesis of core LPS are shown at their approximate site of action (solid line). The sites, which are variably substituted or still under investigation, are indicated by dashed lines. The abbreviations in this figure are as follows: GlcNac, N-Acetylglucosamine; Glc, glucose; Hep, heptose; Gal, galactose; P, phosphate; PPEtn, phosphoethanolamine; KDO, 2-keto-3-deoxyoctonate. It should be noted that *E. coli* K12 does not possess O-Ag.

Table 1. Bacterial strains and cell lines used in this study

Strains	Genotypes (phenotypes)	Refs
<i>E. coli</i> K 12		
CS180	WT (rough)	[29, 53, 54]
CS1861	CS180-O antigen (smooth)	
<i>Y. pseudotuberculosis</i>		
Y 1	O:1a, WT expressing invasin but with PYV plasmid naturally cured (smooth)	[30–32, 55]
<i>S. sonnei</i>		
Ss-WT	WT (smooth)	This study
Ss-rough	without 210 kb virulence plasmid (rough)	This study
Rough O ⁺	<i>S. sonnei</i> -rough with pSS37 (smooth)	This study
Cell lines		
Characteristics		
CHO-NEO cells	Control cell line, which expresses the neomycin resistance gene only	
CHO-mSIGNR1	Generated by transfecting CHO cells with mSIGNR1 cDNAs for stable surface expression	
CHO-hDC-SIGN	Generated by transfecting CHO cells with hDC-SIGN cDNAs for stable surface expression	
CHO-hLangerin	Generated by transfecting CHO cells with hLangerin cDNAs for stable surface expression	

involved in invasion [13]. However, the large plasmid of *S. sonnei* is unstable and easily lost. Why does *S. sonnei* carry genes that control the synthesis of the somatic antigen and the expression of virulence on an unstable plasmid, and what are selective pressures to maintain the virulent state [14]? Maybe, *S. sonnei* has evolved to lose the virulence plasmid as an advantage during infection [15].

Rough Gram-negative bacteria bear shortened LPSs – referred to as lipooligosaccharides (LOS) – for which the oligosaccharide core is exposed to the extracellular environment [9]. Previous studies suggested that by using the LPS core, Gram-negative bacteria can interact with human Langerin (CD207) and human DC-SIGN (CD209), expressed on antigen-presenting cells (APCs) [16–22]. It is well-documented that human DC-SIGN is also a receptor for HIV GP-120 that

hijacks DC-SIGN to be captured by APCs and trafficked to target cells such as CD4 lymphocytes [23–25].

Shigella initially crosses the epithelial layer of the colorectal mucosa via M cells. M cells are capable of transcytosing luminal antigens into the subepithelial space where gut-associated lymphoid tissue and/or APCs, such as dendritic cells (DCs) and macrophages, are located [26]. The APCs of colonic and rectal mucosa can act as ‘Trojan horses’ allowing crossing of the intestinal epithelial barrier [27, 28].

In this study, we investigated if *S. sonnei* rough, rather than smooth, strains interacted with human DCs from guts, murine primary macrophages and the CHO cell lines that express C-type lectins of human CD207, human CD209a and mouse CD209b.

METHODS

Bacterial strains

Bacterial strains used in this study are listed in Table 1. *E. coli* K12 strain CS180 contains core oligosaccharides but lacks the O-antigen. CS1861 is the strain of CS180 harbouring pSS37, a plasmid containing all genes necessary for expression of the *Shigella dysenteriae* serotype 1 O-antigen [29]. *E. coli* strains were cultured on Luria–Bertani (LB) agar at 37°C overnight. *Y. pseudotuberculosis* (Y1) is a serotype O:1a strain, lacking the virulence plasmid (pYV); the strain was obtained from the Centers for Disease Control (GA, USA), and used as a control strain for invasion since this bacterium invades almost all epithelial cell lines via an invasin-integrin interaction [30–32]. This strain has been used as a positive invasion control previously and was cultured on LB agar at 26°C overnight [33]. *S. sonnei*, a clinical strain isolated from a patient with dysentery in Hubei Provincial Center for Disease Control and Prevention, Wuhan, PR China, was incubated in tryptic soy broth (TSB) at 37°C overnight. *S. sonnei* rough strain without the 210 kb virulence plasmid was obtained from TS broth containing 0.6% yeast extract with 1.5% agar and 0.003% Congo red [15, 34]. Rough O⁺ is a derivative of *S. sonnei* rough strains that harbour pSS37, a plasmid containing all of the genes necessary for expression of the *S. dysenteriae* serotype 1 O-antigen [29]. The three *S. sonnei* strains are isogenic. *S. sonnei* rough and rough O⁺ strains were cultured on LB agar with the corresponding antibiotic at 37°C overnight.

Cell lines

Cell lines used in this study are listed in Table 1. CHO-mSIGNR1, CHO-hDC-SIGN and CHO-hLangerin cell lines were generated by transfecting CHO cells with corresponding C-type lectin cDNAs. Transfection was followed by G418 (1.5 mg ml⁻¹) selection and screening for stable surface expression [35]. CHO-NEO cells were used as a control cell line that expresses the neomycin resistance gene only.

LPS isolation and SDS-PAGE silver staining

The LPS extracts were isolated using the Lipopolysaccharide Extraction Kit (iNtRON Biotechnology, Korea),

performed according to the manufacturer's instructions. The *E. coli* strains CS180 and CS1861, which show rough LPS (without O-antigen) and smooth LPS (with O-antigen), respectively, were used as control strains. After purification, the LPS extracts were analysed by 12% Bis-Tris SDS-PAGE and silver stained using the SilverQuest Silver Staining Kit (Invitrogen).

Isolation of mouse peritoneal macrophages

The experiments for isolating mouse peritoneal macrophages have been described previously [20]. After the mice were euthanized, abdomens were immediately exposed, cleaned with 75% ethanol and opened with scissors; 5 ml of RPMI was injected into the intraperitoneal cavity. The mouse abdomen was gently massaged for 2 min and then lavage fluid was collected. The suspension containing the macrophages was seeded onto six-well plates, in which each well contained a 1.5 cm diameter glass cover-slide, and placed in a CO₂ incubator for 1.5 h. The cell layers were washed three times to remove non-adherent cells. Macrophages were then removed from the plastic surface by incubating with citrate saline and re-seeded for interaction assays.

Isolation of human gut DCs

Human intestinal segments were obtained from patients who were undergoing gastrointestinal surgery and provided informed consent. Purification of gut DCs has been described previously [22, 36]. Samples were collected in ice-cold Dutch modification of RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, gentamicin (25 µg ml⁻¹), and penicillin/streptomycin (100 U ml⁻¹). The samples were incubated for 20 min at room temperature in calcium and magnesium-free HBSS containing 1 mM DTT. To remove the epithelium, biopsies were transferred to HBSS containing 1 mM EDTA and incubated for 30 min on a shaker at 37°C. To continue the isolation of gut DCs, the tissue was digested with 1 mg ml⁻¹ collagenase D in HEPES-buffered RPMI 1640 containing 20 µg ml⁻¹ DNase I and 2% FCS at 37°C on a shaker for 90–180 min. Mononuclear cells were separated (650 g, 20 min, room temperature) on Ficoll-Paque and washed in complete medium. The isolated cells were labelled with anti-CD11c⁺ (Biolegend) and anti-hDC-SIGN antibodies (Pharmingen) and then examined by flow cytometry.

Invasion assays

The invasion assays have been described previously [37]. Briefly, host cells (CHO, hLangerin, mSIGNR1 and hDC-SIGN) were plated in a cell culture flask. The degree of cell fusion was 80%. The cells were suspended in RPMI medium supplemented with 2% foetal calf serum at a concentration of 1×10⁵ ml⁻¹ and were added to 24-well plates; 12–24 h later, cells were washed three times. The 500 µl RPMI medium supplemented with 2% foetal calf serum was added to 24-well plates. After the addition of 50 µl of bacterial suspensions at

a concentration of 5×10⁶ c.f.u. ml⁻¹, cells were incubated for 2 h at 37°C in the presence of 5% CO₂.

To determine the internalization of bacteria, gentamicin – which kills extracellular bacteria but cannot penetrate host cells – was added to each well at a final concentration of 100 µg ml⁻¹ and the cultures were incubated for 60 min. The cells were washed three times to remove the gentamicin. Cells were suspended in phosphate-buffered saline containing 0.25% Triton, after which the cells were diluted and plated on LB plates with corresponding antibiotics. The level of internalization of bacteria in the host cells was calculated by determining the c.f.u. recovered from lysed cells. All experiments were performed in triplicate, and the data are expressed as means±standard errors.

For the inhibition assay, the concentration of cells was 2×10⁵ ml⁻¹. Anti-mSIGNR1 (5 µg ml⁻¹) antibody, anti-hLangerin (5 µg ml⁻¹) antibody (Pharmingen), and mannan (500 µg ml⁻¹) (Sigma-Aldrich) were added 20 min before the addition of bacteria. The concentrations used were determined based on our preliminary data and were selected based on the fact that, at these concentrations, the compounds exerted no effects on the survival of bacteria or host cells, as previously shown [17, 18].

Animal assays

All animal experiments were carried out in strict accordance with the Institutional Animal Care and Use Committees (IACUCs) and Institutional Review Board (IRB) of Tongji Hospital, Tongji Medical College, PR China. C57BL/6 female mice at the age of 5–6 weeks were purchased from Wuhan University Animal Center. Mice were housed in animal facilities at Tongji hospital in direct accordance with guidelines drafted by the Animal Care Committees of Tongji Hospital. For bacterial infection, three groups (six mice/group) of C57BL/6 mice were infected with bacteria (1×10⁶ c.f.u.) treated with corresponding antibiotics through an intraperitoneal route to observe dissemination.

To confirm bacterial colonization in infected tissues, spleens and MLNs were collected after 24 h from intraperitoneally challenged mice. Tissues were removed and vigorously washed in PBS with gentamicin (100 µg ml⁻¹) to remove bacteria that were simply attached but had not invaded the tissues [38]. Tissues were then mechanically homogenized in 1 ml of 1% Triton, diluted, and plated onto plates containing antibiotics. Colonies were counted after 18 h of culture at 37°C.

Statistical analyses

All statistical analyses were completed using Prism software, version 6 (Graph Pad, San Diego, CA, USA). Statistical significance was assessed using Student's *t*-test for the univariate analysis of two sets of data and two-way ANOVA for multiple comparisons. *P* < 0.05 was considered statistically significant.

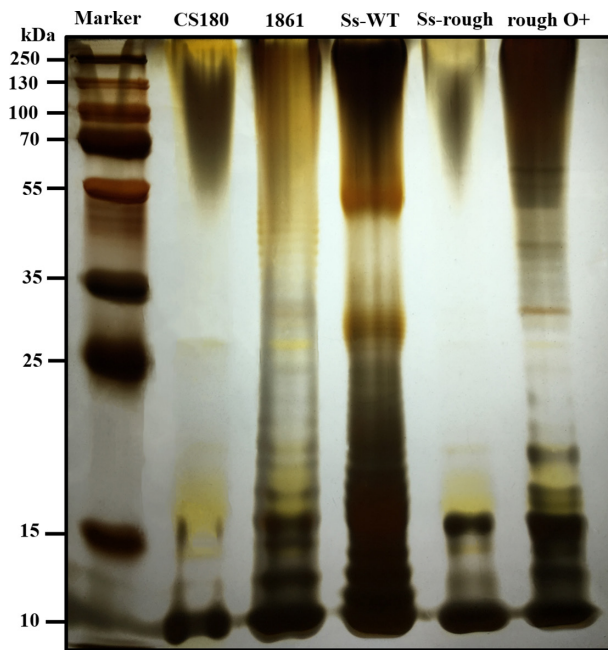


Fig. 2. Examination of O-antigen expression of *S. sonnei* WT and its rough strain. Silver staining of the LPS of *S. sonnei* strains. Controls: *E. coli* strains CS180 and CS1861, which show rough LPS (without O-antigen) and smooth LPS (with O-antigen), respectively. *S. sonnei* strains: Ss-WT has the virulence plasmid to express the O-antigen. Ss-rough and rough O+, which show the rough LPS (without O-antigen) and smooth LPS (with O-antigen), respectively.

RESULTS

S. sonnei without the virulence plasmid did not express the O-antigen

SDS-PAGE silver staining was used to examine the O-antigen expression of *S. sonnei* WT and its rough strain. The *E. coli* strains K-12 CS180 (lacking the O-antigen) and CS1861 (CS180 expressing an O-antigen) were used as controls [29]. As shown in Fig. 2, the LPS extract of *S. sonnei* WT had a typical LPS ladder with a predominant chain length of 20 to 25 O-Ag repeating units; *S. sonnei* rough strain (lacking the O-antigen-encoding virulence plasmid) had only the low molecular weight band corresponding to the LPS core-lipid A moieties. For rough O+, which harboured the pSS37 derivative of CS1861, the O-Ag repeating units were identical to CS1861.

Rough *S. sonnei* invades human DCs and mouse macrophages

We examined the ability of *S. sonnei* WT and its rough strain to invade DCs. The *E. coli* strains K-12 CS180 (an avirulent strain with the core LPS exposed) and CS1861 (CS180 expressing an O-antigen) were used as controls [17, 39]. Results from the gentamicin protection assays (Fig. 3a, b) showed that all of these *S. sonnei* strains were phagocytosed by DCs to some extent, and *S. sonnei* rough strains were taken up more than WT. Previously, it has been reported that the central hydrophobic portion of IpaC on virulence plasmids, the membrane-spanning domain, was critical for entry of *Shigella* into macrophages [40], suggesting that *S.*

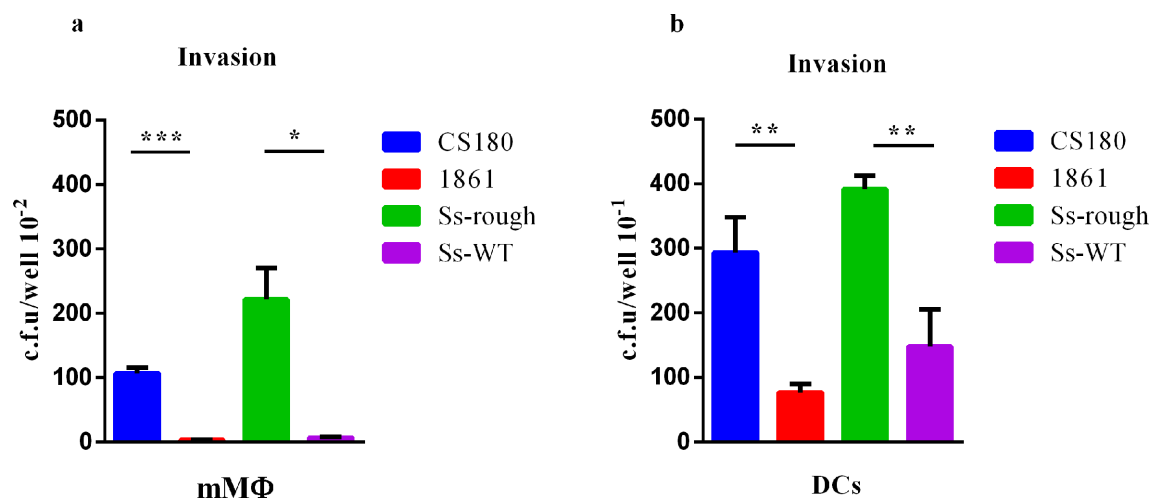


Fig. 3. Human gut dendritic cells (DCs) and mouse macrophages (mMΦ) phagocytose the rough strains *S. sonnei*. Gentamicin protection assays were used to determine the invasion rates of two sets of the Gram-negative bacteria *S. sonnei* (Ss-WT, Ss-rough and rough O+) and *E. coli* K-12 (CS180 and CS1861) into mMΦ (a) or DCs (b). The number of phagocytosed bacteria was determined by counting c.f.u. recovered following gentamycin treatment. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

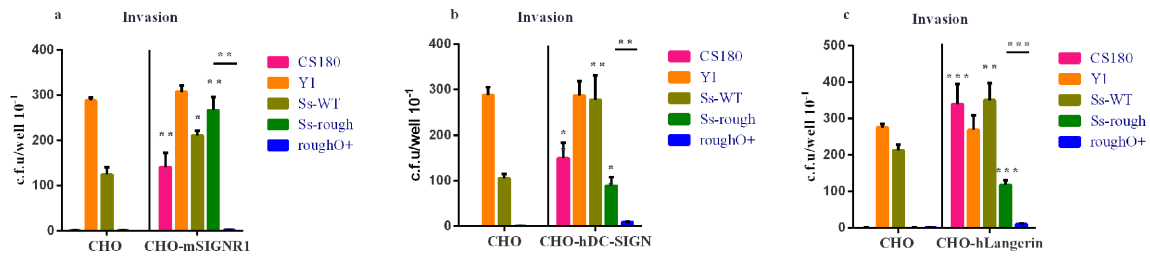


Fig. 4. Phagocytosis of *S. sonnei* strains with transfecting CHO cells. The phagocytosis of two sets of bacteria *E. coli* K-12 (CS180 and CS1861) and *S. sonnei* (Ss-WT, Ss-rough and rough O+) by CHO-mSIGNR1 (a), CHO-hDC-SIGN (b) and CHO-hLangerin (c) cells were analysed. Bacteria and CHO transfectants were incubated together for 2 h, and extracellular bacteria were killed with 100 µg ml⁻¹ (final concentration) gentamicin. CHO-NEO cells were used as the negative control cell line. The number of phagocytosed bacteria was determined by counting c.f.u. recovered following gentamicin treatment. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

sonnei rough strains use different invasive mechanisms to hijack DCs.

CD209 and CD207 are receptors for phagocytosis of rough *S. sonnei*

To further study the interaction of DCs with *S. sonnei* rough strains, stably transfected mSIGNR1/hDC-SIGN/hLangerin CHO cell lines were used to test their ability to bind and internalize *S. sonnei* rough strains. *Y. pseudotuberculosis* (Y1) was used as a control since it invaded almost all epithelial cell lines via an invasion-integrin interaction [18, 31, 33]. Results showed that both CS180 and *S. sonnei* rough strains effectively invaded CHO-mSIGNR1 (Fig. 4a), CHO-hDC-SIGN (Fig. 4b) and CHO-hLangerin (Fig. 4c), but not the CHO. This demonstrated that *S. sonnei* rough strains interacted with mSIGNR1, hDC-SIGN and hLangerin, as the *S. sonnei* rough-mediated invasion was also blocked by the expression of O-antigen.

Inhibition of receptor-mediated phagocytosis of *S. sonnei* rough strains by an anti-mSIGNR1/anti-hLangerin antibody

To verify specific interactions of *S. sonnei* rough and hCD207/CD209 receptors, anti-mSIGNR1/anti-hLangerin antibodies and mannan (an antagonist of mannose receptors) were used. As shown in Fig. 5, when antibodies were applied, the phagocytosis of *S. sonnei* rough strains by transfected CHO cell lines was significantly reduced. This suggests that the mSIGNR1 and hLangerin have a role in the interaction between DCs and *S. sonnei* rough strains. Mannan still inhibited the interactions of CS180 with CD209 and hCD207 (Fig. 5a), as shown in our previous publications [18–20]. However, mannan did reduce the interaction of *S. sonnei* rough strains and CD209, but not the hCD207 receptor (Fig. 5b), suggesting that in addition to the LPS core other sugar residues exposed on the surfaces of *S. sonnei* rough strains may also mediate their interactions.

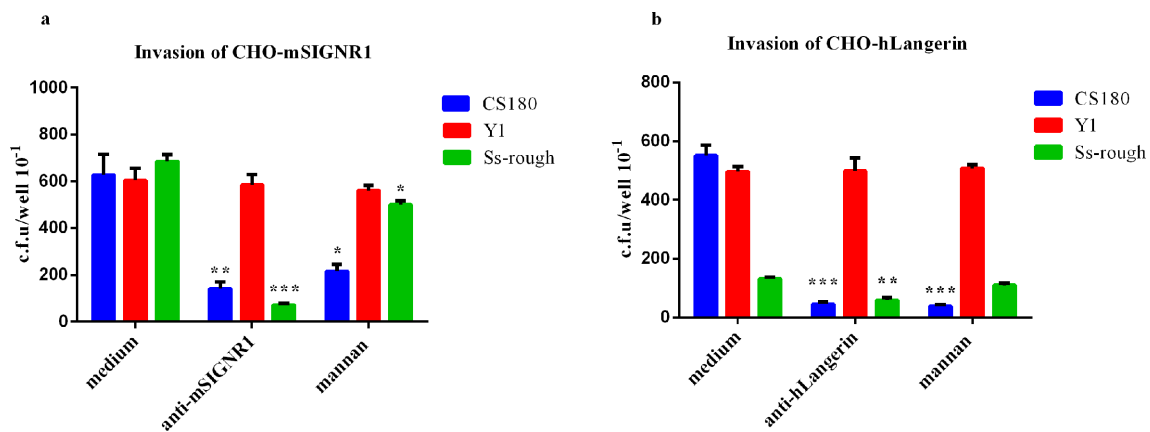


Fig. 5. Inhibition of receptor-mediated phagocytosis of *S. sonnei* rough strain by antibodies. *S. sonnei* rough strain cultured at 37 °C was incubated with CHO-mSIGNR1 (a) and CHO-hLangerin (b) for 2 h in the presence or absence of anti-mSIGNR1, anti-hLangerin antibodies and mannan. All reagents were added to the media 20 min before the addition of bacteria. The phagocytosis rate of Ss-rough was determined by the recovery of bacteria following gentamicin treatment. *Y. pseudotuberculosis* serotype O:1a was used as a control strain that shows core-independent invasion of CHO cells. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

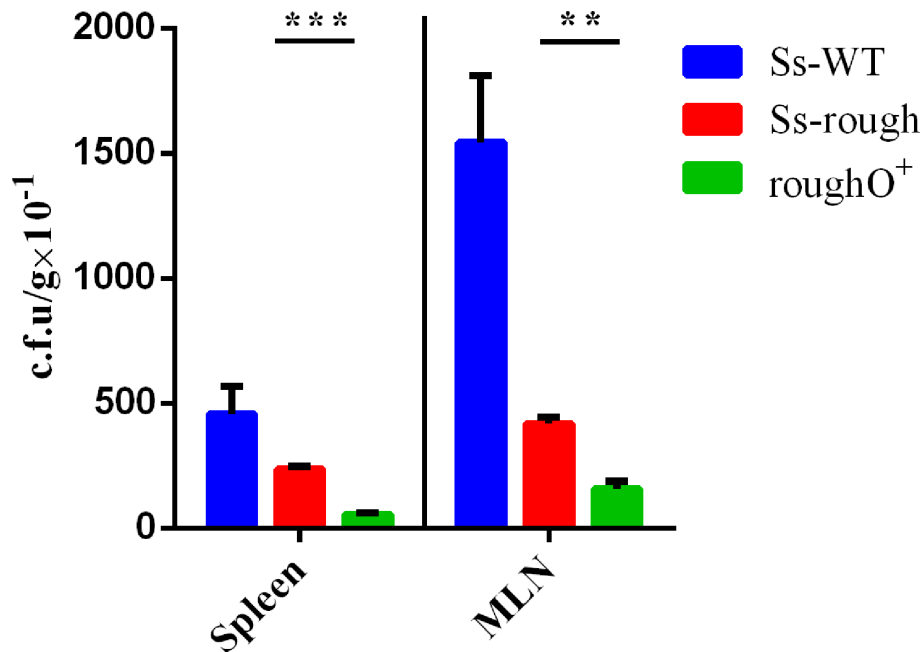


Fig. 6. *S. sonnei* strains disseminate in C57BL/6 female mice after intraperitoneal injection. Ss-WT, Ss-rough and rough O⁺ strains were inoculated in mice following the procedures described in Methods. After 24 h, the mice were killed, and the spleens and MLNs were separated, homogenized and spread on corresponding plates. The dissemination rate represents the c.f.u. recovered from spleens and MLNs. ***P* < 0.01; ****P* < 0.001.

Expression of O-antigen reduces the ability of *S. sonnei* rough strain to be disseminated to mesenteric lymph nodes and spleens

To determine whether *S. sonnei* can be disseminated to local lymph nodes and spleens, mice were infected via the intraperitoneal route. Fig. 6 shows that all *S. sonnei* WT, rough and rough O⁺ strains were isolated in spleens and MLNs, but *S. sonnei* rough strains were recovered from spleens and MLNs in higher numbers than rough O⁺ strains, indicating that the expression of O-antigen reduced the ability of *S. sonnei* to be disseminated. Most likely, because of the invasive genes on virulence plasmids, *S. sonnei* WT had a strong ability to be disseminated.

DISCUSSION

C-type lectins have been studied for their interactions with bacteria, viruses and parasites [41–43]. Both DC-SIGN and Langerin recognize carbohydrate structures with high mannose specificities, therefore, both bind HIV-1 gp120 [44]. Langerin induces the formation of the Birbeck granules in Langerin⁺ cells, and mediates HIV-1 degradation, whereas DC-SIGN mediates HIV-1 transmission [45, 46]. DC-SIGN can bind and readily internalize rough *E. coli*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *S. typhimurium*, *Y. pestis* and *Y. pseudotuberculosis* [17, 18, 20, 39, 47–49]. This CD209-LPS core leads to bacterial dissemination and persistent infection in general [19–22]. We, however, found that DC-SIGN/Langerin mediated the phagocytosis of *S. sonnei*

rough strains, which was not able to cause disease after losing the virulence plasmid [14].

This virulence plasmid is essential for *S. sonnei* pathogenicity [50]. Specifically, infection and dissemination are tightly orchestrated by the IpaB and VirG proteins encoded on the virulence plasmid [51]. After losing the virulence plasmid, *S. sonnei* rough strains fail to escape from macrophages [40], and the CD209/hCD207-*S. sonnei* rough interactions most likely result in the clearance of rough *S. sonnei* during infection. As shown in Fig. 6, immune organs, such as spleens and MLNs, displayed higher levels of *S. sonnei* rough internalization compared to rough O⁺. Functioning as the shield for the O-antigen, it appears that recovery of rough O⁺ bacteria from spleens and MLNs is seen as an indication of partial virulence.

However, a recent paper indicated that deletion of toxin-antitoxin systems in the evolution of *S. sonnei* as a host-adapted pathogen reduces the metabolic burden for *S. sonnei* growth, reflecting the ongoing transition of *S. sonnei* into an obligate pathogen that is less dependent on survival outside a mammalian host than other species of *Shigella* [15]. Moreover, intracellular *Shigella* can remodel its LPS, dampen the innate immune recognition, and evade inflammasome activation [1]. Recent studies by Lugo-Villarino *et al.* demonstrated that DC-SIGN has an anti-inflammatory role in macrophages in response to pathogens [52]. Therefore, based on the CD209/hCD207-*S. sonnei* rough interactions – most likely result in the clearance of rough *S. sonnei* – we hypothesize that *S. sonnei* rough strains may provide for less immune

system-based killing for losing the plasmid during infection (which contributes to *S. sonnei* WT pathogenicity). Future studies may focus on verifying the mechanisms of immune system-based killing for losing the plasmid and helping the survival of WT *S. sonnei*.

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Author contributions

B.C.W., H.H.D. and T. C. designed the experiment and scrutinized the manuscript. B.C.W., N.A.O. and J.M.T. performed the experiments. Y.M.Z., Y.X.H., W.J.L., Y.X., X.X.H., Z.Y.S., Z.J.C., J.C.Q., C.G.P. and A.Y.L. contributed reagents, materials and analysis tools. B.C.W., Y.M.Z., Y.X.H., Y.X., Y.L., H.H.D. and T.C. analysed the data. B.C.W., J.D.K., H.H.D. and T.C. were involved in drafting the manuscript. The authors were given final approval of the version to be published and agreed to be accountable for all aspects of the work.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

All animal procedures and human experiments were carried out in strict accordance with the Institutional Animal Care and Use Committees (IACUCs) and Institutional Review Board (IRB) of Tongji Hospital, Tongji Medical College, China. The handling of the mice and all experimental procedures were specifically approved for this study by the Medical Ethics Committee of Tongji Hospital and were conducted in accordance with the institutional guidelines (IRB ID: TJ-A20141220 for animal experiments and TJC20140113 for human experiments). All procedures on mice were performed under sodium pentobarbital anaesthesia, and all efforts were made to minimize suffering. And all the volunteers involved in the experiment have signed informed consent.

References

- Paciello I, Silipo A, Lembo-Fazio L, Curcurù L, Zumsteg A et al. Intracellular *Shigella* remodels its LPS to dampen the innate immune recognition and evade inflammasome activation. *Proc Natl Acad Sci U S A* 2013;110:E4345–E4354.
- Anderson M, Sansonetti PJ, Marteyn BS. *Shigella* diversity and changing landscape: insights for the twenty-first century. *Front Cell Infect Microbiol* 2016;6:45.
- Kahsay AG, Muthupandian S. A review on sero diversity and antimicrobial resistance patterns of *Shigella* species in Africa, Asia and South America, 2001–2014. *BMC Res Notes* 2016;9:422.
- Gu B, Cao Y, Pan S, Zhuang L, Yu R et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of *Shigella* between Europe-America and Asia-Africa from 1998 to 2009. *Int J Antimicrob Agents* 2012;40:9–17.
- The HC, Thanh DP, Holt KE, Thomson NR, Baker S. The genomic signatures of *Shigella* evolution, adaptation and geographical spread. *Nat Rev Microbiol* 2016;14:235.
- Anderson MC, Vonaesch P, Saffarian A, Marteyn BS, Sansonetti PJ. *Shigella sonnei* encodes a functional T6SS used for inter-bacterial competition and niche occupancy. *Cell Host Microbe* 2017;21:769–776.
- Phalipon A, Sansonetti PJ, Armelle P, SP J. *Shigella's* ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? *Immunol Cell Biol* 2007;85:119–129.
- Shepherd JG, Wang L, Reeves PR. Comparison of O-antigen gene clusters of *Escherichia coli* (*Shigella*) *sonnei* and *Plesiomonas shigelloides* O17: *sonnei* gained its current plasmid-borne O-antigen genes from *P. shigelloides* in a recent event. *Infect Immun* 2000;68:6056–6061.
- Holst O. The structures of core regions from enterobacterial lipopolysaccharides - an update. *FEMS Microbiol Lett* 2007;271:3–11.
- Kalynych S, Morona R, Cygler M. Progress in understanding the assembly process of bacterial O-antigen. *FEMS Microbiol Rev* 2014;38:1048–1065.
- Murray GL, Attridge SR, Morona R. Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz. *Mol Microbiol* 2003;47:1395–1406.
- Murray GL, Attridge SR, Morona R. Altering the length of the lipopolysaccharide O antigen has an impact on the interaction of *Salmonella enterica* serovar *typhimurium* with macrophages and complement. *J Bacteriol* 2006;188:2735–2739.
- Jiang Y, Yang F, Zhang X, Yang J, Chen L et al. The complete sequence and analysis of the large virulence plasmid pSS of *Shigella sonnei*. *Plasmid* 2005;54:149–159.
- Sansonetti PJ, Kopecko DJ, Formal SB. *Shigella sonnei* plasmids: evidence that a large plasmid is necessary for virulence. *Infect Immun* 1981;34:75–83.
- McVicker G, Tang CM. Deletion of toxin-antitoxin systems in the evolution of *Shigella sonnei* as a host-adapted pathogen. *Nat Microbiol* 2016;2:16204.
- Zhang P, Schwartz O, Pantelic M, Li G, Knazze Q et al. DC-SIGN (CD209) recognition of *Neisseria gonorrhoeae* is circumvented by lipooligosaccharide variation. *J Leukoc Biol* 2006;79:731–738.
- Zhang P, Snyder S, Feng P, Azadi P, Zhang S et al. Role of N-acetylglucosamine within core lipopolysaccharide of several species of gram-negative bacteria in targeting the DC-SIGN (CD209). *J Immunol* 2006;177:4002–4011.
- Zhang P, Skurnik M, Zhang SS, Schwartz O, Kalyanasundaram R et al. Human dendritic cell-specific intercellular adhesion molecule-grabbing nonintegrin (CD209) is a receptor for *Yersinia pestis* that promotes phagocytosis by dendritic cells. *Infect Immun* 2008;76:2070–2079.
- Yang K, Park CG, Cheong C, Bulgheresi S, Zhang S et al. Host Langerin (CD207) is a receptor for *Yersinia pestis* phagocytosis and promotes dissemination. *Immunol Cell Biol* 2015;93:815–824.
- He Y-X, Ye C-L, Zhang P, Li Q, Park CG et al. *Yersinia pseudotuberculosis* exploits CD209 receptors for promoting host dissemination and infection. *Infect Immun* 2019;87:e00654–18.
- Yang K, He Y, Park CG, Kang YS, Zhang P et al. *Yersinia pestis* interacts with SIGNR1 (CD209b) for promoting host dissemination and infection. *Front Immunol* 2019;10:96.
- Ye C, Li Q, Li X, Park CG, He Y et al. *Salmonella enterica* serovar *typhimurium* interacts with CD209 receptors to promote host dissemination and infection. *Infect Immun* 2019;87:e00100–00119.
- Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* 2000;100:587–597.
- Engering A, Van Vliet SJ, Geijtenbeek TB, Van Kooyk Y. Subset of DC-SIGN(+) dendritic cells in human blood transmits HIV-1 to T lymphocytes. *Blood* 2002;100:1780–1786.
- McDonald D, Wu L, Bohks SM, KewalRamani VN, Unutmaz D et al. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* 2003;300:1295–1297.
- Nhieu GT, Sansonetti PJ. Mechanism of *Shigella* entry into epithelial cells. *Curr Opin Microbiol* 1999;2:51–55.
- Schroeder GN, Hilbi H. Molecular pathogenesis of *Shigella* spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clin Microbiol Rev* 2008;21:134–156.

28. Sansonetti PJ. Rupture, invasion and inflammatory destruction of the intestinal barrier by *Shigella*, making sense of prokaryote-eukaryote cross-talks. *FEMS Microbiol Rev* 2001;25:3–14.
29. Klena JD, Ashford RS, Schnaitman CA. Role of *Escherichia coli* K-12 *rfa* genes and the RFP gene of *Shigella dysenteriae* 1 in generation of lipopolysaccharide core heterogeneity and attachment of O antigen. *J Bacteriol* 1992;174:7297–7307.
30. Rosqvist R, Skurnik M, Wolf-Watz H. Increased virulence of *Yersinia pseudotuberculosis* by two independent mutations. *Nature* 1988;334:522–525.
31. Isberg RR, Leong JM. Multiple beta 1 chain integrins are receptors for invasins, a protein that promotes bacterial penetration into mammalian cells. *Cell* 1990;60:861–871.
32. Skurnik M, Peippo A, Ervelä E. Characterization of the O-antigen gene clusters of *Yersinia pseudotuberculosis* and the cryptic O-antigen gene cluster of *Yersinia pestis* shows that the plague bacillus is most closely related to and has evolved from *Y. pseudotuberculosis* serotype O:1b. *Mol Microbiol* 2000;37:316–330.
33. Chen T, Belland RJ, Wilson J, Swanson J. Adherence of pilus- Opa+ gonococci to epithelial cells in vitro involves heparan sulfate. *J Exp Med* 1995;182:511–517.
34. Qadri F, Hossain SA, Ciznár I, Haider K, Ljungh A et al. Congo red binding and salt aggregation as indicators of virulence in *Shigella* species. *J Clin Microbiol* 1988;26:1343–1348.
35. Kang YS, Yamazaki S, Iyoda T, Pack M, Bruening SA et al. Sign-R1, a novel C-type lectin expressed by marginal zone macrophages in spleen, mediates uptake of the polysaccharide dextran. *Int Immunol* 2003;15:177–186.
36. Bell SJ, Rigby R, English N, Mann SD, Knight SC et al. Migration and maturation of human colonic dendritic cells. *J Immunol* 2001;166:4958–4967.
37. Chen T, Grunert F, Medina-Marino A, Gotschlich EC. Several carcinoembryonic antigens (CD66) serve as receptors for gonococcal opacity proteins. *J Exp Med* 1997;185:1557–1564.
38. Yang JY, Lee SN, Chang SY, Ko H-J, Ryu S et al. A mouse model of shigellosis by intraperitoneal infection. *J Infect Dis* 2014;209:203–215.
39. Klena J, Zhang P, Schwartz O, Hull S, Chen T. The core lipopolysaccharide of *Escherichia coli* is a ligand for the dendritic-cell-specific intercellular adhesion molecule nonintegrin CD209 receptor. *J Bacteriol* 2005;187:1710–1715.
40. Kuwae A, Yoshida S, Tamano K, Mimuro H, Suzuki T et al. *Shigella* invasion of macrophage requires the insertion of IpaC into the host plasma membrane. Functional analysis of IpaC. *J Biol Chem* 2001;276:32230–32239.
41. Mayer S, Raulf MK, Lepenius B. C-Type lectins: their network and roles in pathogen recognition and immunity. *Histochem Cell Biol* 2017;147:223–237.
42. Preza GC, Tanner K, Elliott J, Yang OO, Anton PA et al. Antigen-presenting cell candidates for HIV-1 transmission in human distal colonic mucosa defined by CD207 dendritic cells and CD209 macrophages. *AIDS Res Hum Retroviruses* 2014;30:241–249.
43. Loukas A, Maizels RM. Helminth C-type lectins and host-parasite interactions. *Parasitol Today* 2000;16:333–339.
44. de Witte L, Nabatov A, Geijtenbeek TB. Distinct roles for DC-SIGN+ dendritic cells and Langerhans cells in HIV-1 transmission. *Trends Mol Med* 2008;14:12–19.
45. Gurney KB, Elliott J, Nassanian H, Song C, Soilleux E et al. Binding and transfer of human immunodeficiency virus by DC-SIGN+ cells in human rectal mucosa. *J Virol* 2005;79:5762–5773.
46. Nasr N, Lai J, Botting RA, Mercier SK, Harman AN et al. Inhibition of two temporal phases of HIV-1 transfer from primary Langerhans cells to T cells: the role of langerin. *J Immunol* 2014;193:2554–2564.
47. Maeda N, Nigou J, Herrmann JL, Jackson M, Amara A et al. The cell surface receptor DC-SIGN discriminates between *Mycobacterium* species through selective recognition of the mannose caps on lipoarabinomannan. *J Biol Chem* 2003;278:5513–5516.
48. Tailleux L, Schwartz O, Herrmann JL, Pivert E, Jackson M et al. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. *J Exp Med* 2003;197:121–127.
49. van Kooyk Y, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. *Nat Rev Immunol* 2003;3:697–709.
50. Sansonetti PJ. Microbes and microbial toxins: paradigms for microbial-mucosal interactions III. Shigellosis: from symptoms to molecular pathogenesis. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G319–323.
51. Bernardini ML, Mounier J, d'Hauteville H, Coquis-Rondon M, Sansonetti PJ. Identification of icsA, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc Natl Acad Sci U S A* 1989;86:3867–3871.
52. Lugo-Villarino G, Troegeler A, Balboa L, Lastrucci C, Duval C et al. The C-Type Lectin receptor DC-SIGN has an anti-inflammatory role in human M(IL-4) macrophages in response to *Mycobacterium tuberculosis*. *Front Immunol* 2018;9:1123.
53. Schnaitman CA, Klena JD. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. *Microbiol Rev* 1993;57:655–682.
54. Klena JD, Schnaitman CA. Function of the *rfa* gene cluster and the *rfe* gene in the synthesis of O antigen by *Shigella dysenteriae* 1. *Mol Microbiol* 1993;9:393–402.
55. Gillenius E, Urban CF. The adhesive protein invasins of *Yersinia pseudotuberculosis* induces neutrophil extracellular traps via β 1 integrins. *Microbes Infect* 2015;17:327–336.

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