Virulence of Broad- and Narrow-Host-Range *Salmonella enterica*
Serovars in the Streptomycin-Pretreated Mouse Model

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*Salmonella enterica* subspecies I serovars are common bacterial pathogens causing diseases ranging from enterocolitis to systemic infections. Some serovars are adapted to specific hosts, whereas others have a broad host range. The molecular mechanisms defining the virulence characteristics and the host range of a given *S. enterica* serovar are unknown. Streptomycin pretreated mice provide a surrogate host model for studying molecular aspects of the intestinal inflammation (colitis) caused by serovar Typhimurium (S. Hapfelmeier and W. D. Hardt, Trends Microbiol. 13:497–503, 2005). Here, we studied whether this animal model is also useful for studying other *S. enterica* subspecies I serovars. All three tested strains of the broad-host-range serovar Enteritidis (125109, 5496/98, and 832/99) caused pronounced colitis and systemic infection in streptomycin pretreated mice. Different levels of virulence were observed among three tested strains of the host-adapted serovar Dublin (SARB13, SD2229, and SD3246). Several strains of host restricted serovars were also studied. Two serovar Pullorum strains (X3543 and 449/87) caused intermediate levels of colitis. No intestinal inflammation was observed upon infection with three different serovar Paratyphi A strains (SARB42, 2804/96, and 5314/98) and one serovar Gallinarum strain (X3796). A second serovar Gallinarum strain (287/91) was highly virulent and caused severe colitis. This strain awaits future analysis. In conclusion, the streptomycin pretreated mouse model can provide an additional tool to study virulence factors (i.e., those involved in enteropathogenesis) of various *S. enterica* subspecies I serovars. Five of these strains (125109, 2229, 287/91, 449/87, and SARB42) are subject of *Salmonella* genome sequencing projects. The streptomycin pretreated mouse model may be useful for testing hypotheses derived from this genomic data.

*Salmonella enterica* infections rank among the most common bacterial infections in animal herds and humans worldwide. *S. enterica* is a gram-negative bacterial species causing diseases ranging from mild self-limiting enterocolitis to severe systemic infections such as typhoid fever. There are six subspecies of *S. enterica*, and the vast majority of human and animal infections are caused by strains belonging to subspecies I. More than 2,000 “serovars” of *S. enterica* can be distinguished which differ in the molecular composition of the flagella and/or the lipopolysaccharide. In spite of their close genetic relationship, there are significant differences in virulence, host adaptation, and host specificity between strains belonging to different *S. enterica* subspecies I serovars (44, 46). A molecular understanding of these differences in virulence is still lacking. Recently, this has inspired a significant number of *Salmonella* genome sequencing projects (four genomes completed; more than five ongoing [http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi; http://www.sanger.ac.uk/Projects/Microbes/; P. Barrow, personal communication]).

There is a large body of epidemiologic data on the host specificity of different *S. enterica* subspecies I serovars. The serovars Paratyphi A, Gallinarum, and Pullorum are restricted to specific hosts: serovar Paratyphi A causes a systemic disease (paratyphoid) in humans (22, 46), serovar Pullorum causes the systemic pullorum disease in poultry; in freshly hatched chicks, serovar Pullorum causes high mortality and also intestinal inflammation (10); serovar Gallinarum causes the severe systemic fowl typhoid disease in poultry and a few other avian species. Experimental evidence suggests that serovars Gallinarum and Pullorum do not cause disease in mice (1). In spite of some elegant experimental work comparing the virulence characteristics of several different serovars (8, 21, 28, 32, 33, 47, 49, 52), the molecular mechanisms responsible for the host restriction of Gallinarum, Pullorum, or Paratyphi A are still poorly understood. A combination of genome analyses of host restricted serovars and studies in suitable animal models may allow deeper insights.

Some *S. enterica* subspecies I serovars show a preference for certain hosts but are not entirely restricted to them, e.g., serovar Dublin is adapted to cattle, where it causes systemic and enteric disease. Infrequently, serovar Dublin causes septicaemia and enteric disease in humans (24). In laboratory settings serovar Dublin was found capable of causing typhoid fever-like infections in mice (1). Several key virulence factors of serovar Dublin have been characterized (50, 55). The identification of further virulence factors will be fueled by the ongoing serovar Dublin genomic sequencing project (P. Barrow, personal communication).

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S. enterica subspecies 1 serovars Typhimurium and Enteritidis infect a broad range of host animals. Interestingly, they cause different diseases in different animal species. In calves, serovar Typhimurium (and rarely Enteritidis [34]) causes enterocolitis, and the animals can succumb to dehydration (15, 42, 43, 51). In newly hatched chicks, serovars Enteritidis and Typhimurium cause systemic disease and diarrhea, whereas older chickens are asymptomatic carriers (2, 3, 6, 54). In immunocompetent humans, serovars Enteritidis and Typhimurium cause localized self-limiting enterocolitis. Systemic disease may develop in immunocompromised individuals (26). Finally, serovars Enteritidis and Typhimurium cause a systemic typhoid fever-like disease (5, 12, 44) in susceptible mouse strains, but no diarrhea. The mechanisms determining which type of disease is caused in which host by serovars Enteritidis and Typhimurium are still poorly understood.

In the case of serovar Typhimurium infections, the intestinal microflora is an important factor (termed “colonization resistance” [48] or “microbial interference” [30]) in determining whether enteric disease can develop or not. This was demonstrated with germfree mice lacking the entire microflora and with streptomycin-pretreated mice which have a severely disrupted intestinal microflora (4, 13, 41). In the absence of an intact intestinal microflora, serovar Typhimurium not only causes the well-known systemic disease but also colonizes the murine cecum and colon and causes pronounced colitis. This streptomycin-pretreated mouse model has proven useful to study key aspects of the molecular pathogenesis of serovar Typhimurium enterocolitis (17–19, 40). Here, we have extended our studies and tested the virulence of several host-restricted and further broad-host-range S. enterica subspecies 1 serovars, including 6 strains whose genomic sequence has been or will soon be completed. Our data establish that the streptomycin-pretreated mouse model is not restricted to serovar Typhimurium. It also can serve as an interesting additional tool for studying tissue colonization and intestinal inflammation by serovars Enteritidis, Dublin, and Pullorum and even by one highly virulent strain of serovar Gallinarum.

**MATERIALS AND METHODS**

**Bacterial strains.** Wild-type strains of *S. enterica* subspecies 1 serovars Typhimurium, Dublin, Paratyphi A, Gallinarum, Pullorum, and Enteritidis were obtained from the indicated sources (Table 1). The wild-type strains of each serovar were made streptomycin resistant through P22 phage transduction of the streptomycin resistance gene (confers high-level streptomycin resistance) from serovar Typhimurium ATCC 9150 and the wild-type strains of each serovar were obtained from Harlan (Horst, The Netherlands). Briefly, for infection experiments, bacteria were washed twice with PBS to verify that genetic manipulation had not affected the growth properties of the gene.

**Gallinarum**

- **X3796**
  - Wild-type isolate
  - R. Curtiss, United States

- **287/91**
  - Wild-type isolate
  - P. Barrow, IAH, United Kingdom

**Pullorum**

- **X5343**
  - Wild-type isolate
  - R. Curtiss, United States

- **449/87**
  - Wild-type isolate
  - P. Barrow, IAH, United Kingdom

**Enteritidis**

- **125109**
  - Wild-type isolate
  - P. Barrow, IAH, United Kingdom

- **5496/98**
  - Wild-type isolate
  - RKI, Wernigerode, Germany

- **832/99**
  - Wild-type isolate
  - RKI, Wernigerode, Germany

### Table 1. Bacterial strains used in this work

<table>
<thead>
<tr>
<th><em>S. enterica</em> serovar and strain</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium TL1344*</td>
<td>Wild-type isolate</td>
<td>42</td>
</tr>
<tr>
<td>SB161</td>
<td>SL1344, ΔinvG</td>
<td>23</td>
</tr>
<tr>
<td>Dublin</td>
<td>SARB collection</td>
<td>9</td>
</tr>
<tr>
<td>SARB13</td>
<td>Wild-type isolate</td>
<td>Tim Wallis, IAH, United Kingdom</td>
</tr>
<tr>
<td>SD2229*</td>
<td>Wild-type isolate</td>
<td>Tim Wallis, IAH, United Kingdom</td>
</tr>
<tr>
<td>SD3246</td>
<td>Wild-type isolate</td>
<td>Tim Wallis, IAH, United Kingdom</td>
</tr>
<tr>
<td>Paratyphi A SARB42*</td>
<td>ATCC 9150, SARB collection</td>
<td>9</td>
</tr>
<tr>
<td>2804/96</td>
<td>Wild-type isolate</td>
<td>RKI, Wernigerode, Germany</td>
</tr>
<tr>
<td>5314/98</td>
<td>Wild-type isolate</td>
<td>RKI, Wernigerode, Germany</td>
</tr>
<tr>
<td>Gallinarum X3796</td>
<td>Wild-type isolate</td>
<td>R. Curtiss, United States</td>
</tr>
<tr>
<td>287/91#</td>
<td>Wild-type isolate</td>
<td>P. Barrow, IAH, United Kingdom</td>
</tr>
<tr>
<td>Pullorum X5343</td>
<td>Wild-type isolate</td>
<td>R. Curtiss, United States</td>
</tr>
<tr>
<td>449/87#</td>
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<td>P. Barrow, IAH, United Kingdom</td>
</tr>
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<td>Enteritidis 125109*</td>
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<td>5496/98</td>
<td>Wild-type isolate</td>
<td>RKI, Wernigerode, Germany</td>
</tr>
<tr>
<td>832/99</td>
<td>Wild-type isolate</td>
<td>RKI, Wernigerode, Germany</td>
</tr>
</tbody>
</table>

* a, Genome sequence completed (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi); #, genome sequence partially available or available in the near future. All strains carry the streptomyein resistance gene and from SL1344. Typing techniques (phage typing, PFGE, ISS200 Southern blot hybridization, and/or plasmid profiling) indicated that all strains selected from one serovar were genetically distinct. SD2229 and SD3246, as well as 125109 and 5496/98, showed identical PFGE patterns and may therefore be closely related or belong to the same epidemic clones.

b IAH, Institute for Animal Health.

mice were pretreated with 20 mg of streptomycin 1 day prior to infection with ca. 5 × 10⁷ CFU of the indicated bacterial strain. At the indicated times postinfection (p.i.), the mice were sacrificed, and we analyzed intestinal inflammation and bacterial loads in the intestinal tract, spleen, and liver (4). Animal experiments were approved by the Swiss authorities and were performed according to the legal requirements.

**Analysis of Salmonella loads in cecal lumen, mesenteric lymph nodes, liver, and spleen.** To analyze colonization, the spleen, liver, and the mesenteric lymph nodes (MLN) were removed aseptically and homogenized in PBS (containing 0.5% Tergitol and 0.5% bovine serum albumin) at 4°C as described previously (4). The bacterial loads were determined by plating samples on MacConkey agar plates containing 50 μg of streptomycin per ml. The minimal detectable level was 10 CFU/organ for the MLN, 20 CFU/organ for the spleen, and 100 CFU/organ for the liver. The bacterial loads in the cecum content were determined by plating. The minimum detectable level was between 67 and 400 CFU per 25- to 150-ng sample of intestinal contents.

**Histological procedures.** Tissue samples were cryo-embedded, stained with hematoxylin and eosin (HE) and evaluated as described recently (4, 40). Briefly, we evaluated (i) submucosal edema (score: 0, no pathological changes; 1, de-
FIG. 1. Virulence of different *S. enterica* serovars in streptomycin pretreated mice. Six (or five) streptomycin-pretreated mice were infected for 3 days with $5 \times 10^7$ CFU of the indicated serovar (Typhimurium SL1344, Dublin SARB13, Paratyphi A SARB42, Pullorum X3543, Gallinarum X3796, and Enteritidis 125109). (A to E) Bacterial loads in the cecal content (A), the liver (B), the spleen (C), and the mLN (D). The dotted line

\[
\begin{array}{cccc}
\text{strain:} & \text{SL1344} & \text{SARB13} & \text{X3796} \\
\text{stat. anal.*:} & -.002 & .002 & .002 & .002 & .03 \\
\end{array}
\]

* vs serovar Typhimurium SL1344

\[
\begin{array}{cccc}
\text{strain:} & \text{SL1344} & \text{SARB13} & \text{X3796} \\
\text{stat. anal.*:} & -.002 & .002 & .002 & .002 & NS \\
\end{array}
\]

* vs serovar Typhimurium SL1344

\[
\begin{array}{cccc}
\text{strain:} & \text{SL1344} & \text{SARB13} & \text{X3796} \\
\text{stat. anal.*:} & -.002 & .002 & .002 & .002 & NS \\
\end{array}
\]

* vs serovar Typhimurium SL1344

\[
\begin{array}{cccc}
\text{strain:} & \text{SL1344} & \text{SARB13} & \text{X3796} \\
\text{stat. anal.*:} & -.006 & .002 & NS & .002 & .002 \\
\end{array}
\]

* vs serovar Typhimurium SL1344
tectable edema [submucosal edema, <10%]; 2, moderate edema [submucosal edema, 10 to 40%]; 3, profound edema [submucosal edema, ≥40%]), (ii) polymorphonuclear leukocyte (PMN) infiltration into the lamina propria (score: 0, fewer than 5 PMN per high-power field; 1, 5 to 20 PMN per high-power field; 2, 21 to 60 PMN per high-power field; 3, 61 to 100 PMN per high-power field; 4, more than 100 PMN per high-power field), (iii) goblet cells (score: 0, more than 28 goblet cells per high-power field; 1, 11 to 28 goblet cells per high-power field; 2, 1 to 10 goblet cells per high-power field; 3, fewer than 1 goblet cell per high-power field), and (iv) epithelial integrity (score: 0, no pathological changes detectable; 1, epithelial desquamation; 2, erosion of the epithelial surface; 3, epithelial ulceration). The combined scores indicated the following conditions: 0, intestine intact without any signs of inflammation; 1 to 2, minimal signs of inflammation (frequently found in the cecum of specific-pathogen-free mice); 3 to 4, slight inflammation; 5 to 8, moderate inflammation; and 9 to 13, profound inflammation.

Statistical analysis. Statistical analyses of the individual pathological scores for submucosal edema, PMN infiltration, loss of goblet cells, and epithelial integrity and of the combined pathological score were performed by using the exact Mann-Whitney U test and the SPSS software, version 11.0, as described previously (4). P values of <0.05 were considered statistically significant. Bacterial colonization was analyzed in a similar manner. To allow statistical analysis of the bacterial loads, the values used for animals that yielded no CFU were set to the “minimal detectable levels” (mLN, 10 CFU; spleen, 20 CFU; liver, 100 CFU; intestinal contents, between 67 and 400 CFU [see above]). After this, the median value was calculated by using Microsoft Excel XP, and a statistical analysis was performed by using the exact Mann-Whitney U test and the SPSS software, version 11.0. P values of <0.05 were considered statistically significant.

RESULTS

Virulence of different S. enterica subspecies 1 serovars in the streptomycin pretreated mouse model. In specific-pathogen-free mice, serovars Enteritidis and Dublin are known to cause typhoid fever-like systemic infections but no intestinal inflammation (1, 44, 45). Moreover, the host-restricted serovars Paratyphi A, Gallinarum, and Pullorum are not thought to cause any disease in mice (1) upon oral inoculation. It was of interest to determine whether or not streptomycin pretreated mice might develop disease in response to any of these serovars. For this purpose, groups of six C57Bl/6 mice (or five mice in the case of serovar Enteritidis) were pretreated with streptomycin and infected intragastrically with ca. 5 × 10^2 CFU of serovars Dublin (SARB13), Paratyphi A (SARB42 = ATCC 9150), Gallinarum (X3796), Pullorum (X3543), and Enteritidis (strain SARB13), and host-restricted serovars Paratyphi A (SARB42) and Gallinarum (X3796) did not cause pronounced intestinal inflammation. With these latter serovars, only low levels of edema, PMN infiltration, and reduction of goblet cell numbers were observed which resemble histopathological findings in healthy specific-pathogen-free mice (4). Thus, Paratyphi A strain SARB42 and Gallinarum strain X3796 are not considered to cause intestinal inflammation.

These results indicated that besides Typhimurium (SL1344), at least some strains of the broad-host-range serovar Enteritidis (125109), the host-adapted serovar Dublin (SARB13), and the host-restricted serovar Pullorum (X3543) are capable of causing intestinal inflammation in the streptomycin pretreated mouse model. The strains of the host-restricted serovars Gallinarum and Paratyphi A that were analyzed in this first exploratory experiment did not cause pronounced intestinal inflammation or systemic infection. Further work was required to determine whether these observations can be generalized to more or all strains of a specific serovar (see below).

Virulence of different isolates from the same serovar. The data above demonstrated that certain strains of broad-host-range (e.g., Enteritidis 125109), host-adapted (e.g., Dublin SARB13), and host-restricted (e.g., Pullorum X3543) S. enterica subspecies 1 serovars can cause colitis in streptomycin-
pretreated mice, whereas others (e.g., Gallinarum) were not (Fig. 1). However, it had remained unclear whether this capacity to cause intestinal inflammation (or not) was attributable to serovar-specific virulence functions. Considering that the assignment of serovars is based on phenotypic properties (lipopolysaccharide and flagellar antigens) and not on phylogenetic relationship, it was conceivable that the virulence might differ significantly between different strains of the same serovar. To test this hypothesis, we compared the virulence of different strains of serovars Dublin (SARB13, SD2229, and SD3246), Gallinarum (X3796 and 287/91), Pullorum (X3543, 449/87) and Enteritidis (125109, 5496/98, and 832/99). This included three strains (SD2229, 287/91, and 449/87) whose genomic sequence will be available in the near future (http://www.sanger.ac.uk/Projects/Salmonella/; P. Barrow, personal communication). Groups of five streptomycin-pretreated

FIG. 2. Cecal inflammation at 3 days p.i. HE-stained cecal tissue sections from six representative animals of the experiment shown in Fig. 1. The cecal tissues were obtained from mice infected with serovar Typhimurium SL1344 (A and D), serovar Dublin SARB13 (B and E), serovar Paratyphi A SARB42 (C and F), serovar Pullorum X3543 (G and J), serovar Gallinarum X3796 (H and K), or serovar Enteritidis 125109 (I and L). Boxes in panels A, B, C, G, H, and I indicate the area shown at the higher magnification. L, intestinal lumen; e, edema; g, goblet cell; sa, submucosa. Magnifications are indicated by the black bars. Scale bars: A, B, C, G, H, and I, 200 μm; D, E, F, J, K, and L, 100 μm.
FIG. 3. Serovar Dublin colitis in the streptomycin-pretreated mice. Five streptomycin-pretreated mice were infected for 3 days with $5 \times 10^7$ CFU of serovar Dublin strains SARB13, SD2229, or SD3246. (A to E) Bacterial loads in the cecal content (A), the liver (B), the spleen (C), and the mLN (D). The dotted line indicates the limit of detection, and the horizontal bars indicate the medians. (E) Histopathological analysis. HE-stained sections of cecal tissue were scored for edema in the submucosa (black bars); PMN infiltration (black dotted bars); reduction in the number of goblet cells (white dotted bars); and desquamation, erosion, and ulceration of the epithelial layer (white bars) (see Materials and Methods). The scores are expressed as stacked vertical bars. Differences in colonization or the total pathological score (sum of the separate scores) were statistically analyzed by using the exact Mann-Whitney U test.
C57BL/6 mice were infected for 3 days with the indicated strains, and we analyzed colonization of the cecal lumen, liver, spleen, and mLN, as well as intestinal inflammation (see Materials and Methods).

**Serovar Dublin.** The virulence of the three serovar Dublin strains (SARB13, SD2229, and SD3246) differed significantly. SD2229 was more efficient than SARB13 and SD3246 at colonizing liver and spleen ($10^7$ versus $10^3$ to $10^6$ CFU/organ; $P = 0.008$; Fig. 3B and C). Furthermore, SD2229 colonized the cecal lumen ($10^7$ CFU/g) and the mLN ($10^3$ CFU) more efficiently than the other two serovar Dublin strains (Fig. 3A and D). Pronounced inflammation was evident in the ceca and colons of mice infected with SD2229 and SARB13 (Fig. 3E; data not shown), whereas SD3246 caused only mild colitis. Clearly, the serovar Dublin strain SD2229 was more virulent in the streptomycin-pretreated mouse model than the other two strains tested. In fact, tissue colonization by SD2229 and the extent of the cecal inflammation were similar to that observed with the serovar Typhimurium (SL1344) control strain (Fig. 1) (19).

**Serovars Gallinarum and Pullorum.** Next, we compared the virulence of two serovar Gallinarum (X3796 and 287/91) and two serovar Pullorum strains (X3543 and 449/87) at 3 days p.i. in the streptomycin-pretreated mouse model. As expected for the fowl-restricted serovars Pullorum and Gallinarum (33), low numbers ($10^2$ to $10^3$ CFU/organ) of serovar Gallinarum (X3796) and Pullorum (X3543 and 449/87) were found in the murine livers and spleens (1, 32). In the animals infected with these strains, the mLN harbored $10^3$ to $10^4$ CFU, and colonization of the cecal lumen was also quite low ($10^4$ to $10^6$ CFU/g, liver ($10^7$ to $10^8$ CFU/organ), spleen ($10^5$ CFU/organ), and mLN ($10^7$ to $10^8$ CFU/organ) with similar high efficiencies (Fig. 6A to D). In fact, colonization by all serovar Enteritidis strains was very similar to that commonly observed with the serovar Typhimurium strain SL1344 (Fig. 1) (19), which served as a control in the present study. Macroscopically, the mLN of all mice from all three groups were swollen, the cecum and the colon were pale, and the cecum was shriveled to a small size and filled with purulent exudates. In line with these observations, histopathological evaluation revealed severe inflammation of the cecum (Fig. 6E) and colon (data not shown) in all animals. Thus, the sequenced strain 125109 seems to represent serovar Enteritidis virulence quite well. And the streptomycin-pretreated mouse model should provide a versatile surrogate host animal model for studying specific details of the serovar Enteritidis-host interaction, which lead to intestinal inflammation.

**Serovar Paratyphi A.** To analyze strain-specific virulence characteristics, we compared the serovar Paratyphi A strain SARB42 with two Paratyphi A patient isolates from Germany (2804/96 and 5314/98; Table 1). Paratyphi A strains 2804/96 and 5314/98 colonized the large intestine of streptomycin pretreated mice more efficiently than SARB42 (Fig. 7). However, bacterial densities were still ~100-fold lower than in serovar Typhimurium- or Enteritidis-infected mice (Fig. 1 and 6). All other virulence parameters (colonization of mLN, spleen, or liver; lack of cecal inflammation) were quite similar between all three Paratyphi A isolates (Fig. 7). These data indicate that “avirulence” in the streptomycin pretreated mouse model may be a common characteristic of serovar Paratyphi A strains.

**Invasion into murine intestinal epithelial cells.** Entry into mucosal tissues is a hallmark of enteric salmonellosis. Invasion of intestinal epithelial and transepithelial transport by dendritic cells have been implicated in penetration of the epithelial barrier by serovar Typhimurium (16, 18, 31, 38). We have performed invasion assays by using cultured murine intestinal epithelial cells (m-ICcl2) (7) to characterize the different serovar Dublin, Paratyphi A, Gallinarum, Pullorum, and Enteritidis strains in more detail. Wild-type serovar Typhimurium SL1344 and a noninvasive isogenic mutant (SB161 and SL1344ΔinvG) (23) served as controls. In line with the mouse virulence data, all three Enteritidis strains were highly invasive (Fig. 8). As expected for nonflagellated Salmonella spp. (14), Gallinarum and Pullorum strains were not highly invasive. Nevertheless, the strain (Gallinarum 287/91) most virulent in the mouse model also showed the highest invasion efficiency among the strains tested (Fig. 8).

Large differences in invasion efficiency were observed between the different Dublin and Paratyphi A strains. It is possible that differences in the effector protein repertoires might play a role in this. To address this, we have compared TTSS-1 and TTSS-2 effector protein genes from Typhimurium LT2, Enteritidis 125109, Paratyphi A SARB42, and Gallinarum 287/91 (data not shown). The large majority of effector protein genes (e.g., sipA, sipB, sopB, sopD, sopE, sopE2, spP, and avrA), including the key effector proteins known to be required for murine serovar Typhimurium colitis (17) were intact in all four Salmonella strains. Interestingly, the sopA gene is only functional in LT2, Enteritidis 125109, and Gallinarum 287/91 but not in Paratyphi A SARB42 (27). SopA has recently been described as an invasion factor for polarized epithelial cells...
FIG. 4. Virulence of Gallinarum and Pullorum strains in streptomycin-pretreated mice. Five streptomycin pretreated mice were infected for 3 days with $5 \times 10^7$ CFU of serovar Gallinarum X3796, Gallinarum 287/91, Pullorum X3543, or Pullorum 449/87. (A to E) Bacterial loads in the cecal content (A), the liver (B), the spleen (C), and the mLN (D). The dotted line indicates the limit of detection, and the horizontal bars indicate the medians. (E) Histopathological analysis. HE-stained sections of cecal tissue were scored for edema in the submucosa (black bars), PMN infiltration (black dotted bars), reduction in the number of goblet cells (white dotted bars), and desquamation, erosion, and ulceration of the epithelial layer (white bars) (see Materials and Methods). The scores are expressed as stacked vertical bars. Differences in colonization or the total pathological score (sum of the separate scores) were statistically analyzed by using the exact Mann-Whitney U test.
serovars. We found that streptomycin-pretreated mice develop any flora do not normally get overt intestinal inflammation from surrogate host model because mice with an intact intestinal microbiota tested did not cause intestinal inflammation.

Mouse model. In contrast, all serovar Paratyphi A strains possessed the capacity to cause colitis in the streptomycin-pretreated mouse model. In the murine host-restricted serovar Gallinarum hardly ever differs from virulence in the mouse model and tissue culture invasiveness. However, it should be noted that the correlation between invasion efficiency in tissue culture and triggering of intestinal inflammation in animal models is only poorly understood. Future work will have to address this issue in more detail.

**DISCUSSION**

It is well established that different serovars of *S. enterica* subspecies 1 can cause diseases ranging for mild self-limiting enterocolitis to life threatening systemic diseases. Some serovars are strictly confined to one specific host species, whereas others have a broad host range. The molecular mechanisms determining host specificity are poorly understood. The same holds true for the molecular mechanisms determining which type of disease is caused in which animal species. In the murine model we have observed that pretreatment with streptomycin can alter the type of disease caused by serovar Typhimurium: mice with a normal intestinal flora develop a typhoid fever-like serovar Typhimurium infection. In contrast, in streptomycin-pretreated mice the systemic infection is accompanied by pronounced intestinal inflammation. This murine serovar Typhimurium colitis model can help to explore molecular mechanisms of the pathogen-host interaction which lead to acute intestinal inflammation (4, 17, 19, 40). In the present study, we have extended our initial observations to other *S. enterica* subspecies 1 serovars. We found that streptomycin-pretreated mice develop pronounced colitis upon infection with different strains of serovar Enteritidis and mild colitis upon infection with different strains of serovar Pullorum. Among the serovars Dublin and Gallinarum strains, we observed strain-specific differences in the capacity to cause colitis in the streptomycin-pretreated mouse model. In contrast, all serovar Paratyphi A strains tested did not cause intestinal inflammation.

Clearly, the streptomycin-pretreated mouse model is a surrogate host model because mice with an intact intestinal microflora do not normally get overt intestinal inflammation from any *S. enterica* serovar. Nevertheless, it should be noted that most of the serovars analyzed are actually capable of causing colitis in at least some of their natural hosts. Serovar Enteritidis is a frequent cause of enterocolitis in humans, serovar Pullorum does cause diarrhea in freshly hatched birds (39, 53), serovar Dublin can cause intestinal inflammation in calves, and human cases of serovar Dublin colitis have also been observed (44, 50). Our observations suggest that the streptomycin-pretreated mouse model provides an interesting new tool for studying virulence factors of serovars Enteritidis, Pullorum, and Dublin which are involved in intestinal inflammation. In these cases it will be of great advantage that the genomes of the murine host and the genomes of the pathogen are (or will soon be) known at the nucleotide level. Both, the host and the pathogen are amenable to efficient genetic manipulation. The use of knockout bacteria and knockout mice (e.g., mice deficient in innate immune response pathways), transgenic animals, and sophisticated tools for studying murine inflammation and immune responses will allow developing working models which can later be tested in the respective natural host species.

In conclusion, we identified some parallels but also clear differences between virulence in the mouse model and tissue culture invasiveness. It should be noted that the correlation between invasion efficiency in tissue culture and triggering of intestinal inflammation in animal models is only poorly understood. Future work will have to address this issue in more detail.
FIG. 6. Virulence of serovar Enteritidis strains in streptomycin-pretreated mice. Five streptomycin-pretreated mice were infected for 3 days with $5 \times 10^7$ CFU of serovar Enteritidis strain 125109, 5496/98, or 832/99. (A to E) Bacterial loads in the cecal content (A), the liver (B), the spleen (C), and the mLN (D). The dotted line indicates the limits of detection, and the horizontal bars indicate the medians. (E) Histopathological analysis. HE-stained sections of cecal tissue were scored for edema in the submucosa (black bars); PMN infiltration (black dotted bars); reduction in the number of goblet cells (white dotted bars); and desquamation, erosion, and ulceration of the epithelial layer (white bars) (see Materials and Methods). The scores are expressed as stacked vertical bars. Differences in colonization or the total pathological score (sum of the separate scores) were statistically analyzed by using the exact Mann-Whitney U test.
FIG. 7. Virulence of serovar Paratyphi A strains in streptomycin-pretreated mice. Five streptomycin-pretreated mice were infected for 3 days with $5 \times 10^7$ CFU of serovar Paratyphi A strain SARB42, 2804/96, or 5314/98. (A to E) Bacterial loads in the cecal content (A), the liver (B), the spleen (C), and the mLN (D). The dotted line indicates the limits of detection, and the horizontal bars indicate the medians. (E) Histopathological analysis. HE-stained sections of cecal tissue were scored for edema in the submucosa (black bars); PMN infiltration (black dotted bars); reduction in the number of goblet cells (white dotted bars); and desquamation, erosion, and ulceration of the epithelial layer (white bars) (see Materials and Methods). The scores are expressed as stacked vertical bars. Differences in colonization or the total pathological score (sum of the separate scores) were statistically analyzed by using the exact Mann-Whitney U test.
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FIG. 8. Invasion assay. Invasion of polarized m-ICc12 cells by different strains of serovar Dublin, Paratyphi A, Gallinarum, Pullorum, and Enteritidis. Typhimurium SL1344 and SB161 (SL1344 ΔinvG) served as a control. The data are normalized to the number of CFU recovered from the wells infected with Typhimurium SL1344. The data are presented as the mean (± the standard deviation) of triplicate results obtained in three independent experiments.

two alternative explanations for the distinct virulence of serovar Gallinarum strains X3796 and 287/91, which we have observed in our study. (i) 287/91 may have acquired additional virulence functions that are normally not present in serovar Gallinarum strains. Experiments in other animal models would be helpful to substantiate this hypothesis. Furthermore, the genomic sequence of this strain that is currently being completed at the Sanger Center (http://www.sanger.ac.uk/Projects/S. Enterica/) may help to identify putative 287/91-specific virulence factors. (ii) Strain X3796 may lack one or more virulence factors. For example, virulence genes might have been disrupted or lost during strain storage. Future work will address this issue. Anyway, our data confirm that virulence characteristics can differ significantly between strains of the same serovar. This should be kept in mind when published data are interpreted and in future studies aimed at identifying serovar-specific virulence factors.

We have demonstrated that the streptomycin-pretreated mouse model can provide a useful additional tool for studying virulence functions of other S. enterica serovars besides Typhimurium. This is of considerable interest because it allows taking advantage of a relatively cheap, well-defined, and genetically amenable animal model for analyzing hypotheses that are being developed based on in silico studies of the ever-increasing number of sequenced Salmonella genomes. The virulence data for a total of six sequenced S. enterica subspecies I strains in the streptomycin-pretreated mouse model provide an excellent starting point for this genome-inspired research in Salmonella pathogenesis.


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