Supplement

Relapse of *S.* Tm^{att} excretion in antibiotic treated LCM mice

Besides gut mucosal immune defense mechanisms, the highly dense and diverse bacterial community in the gut (the microbiota; >500 different species (2, 37)) plays a key role for prohibiting with pathogen growth in the gut lumen. This phenomenon is referred to as colonization resistance and efficiently prevents infections by *Clostridium difficile*, *Salmonella* spp. and other pathogenic bacteria (75). In addition, the microbiota play a crucial role in *S.* Typhimurium clearance after primary infection (22). Antibiotic treatment disturbs the natural microbiota, decreases colonization resistance and represents a known risk factor for *Salmonella* infections in humans and mice (4, 7, 16, 19, 70). It has been shown that after antibiotic therapy some microorganisms are lost from the gut flora and do not return to pretreatment levels (38, 39). This removal is not limited to microorganisms affected by the antibiotic itself. Some commensals are thought to be lost in an 'indirect' fashion, i.e. due to co-dependence among the members of this community. Moreover drastic variations of the species composition can be observed between different individuals (73). Hence a complex microbiota might prevent a relapse of pathogen growth in the gut (i.e. shedding) after antibiotic therapy and thus prevent the emergence of long term asymptomatic excretors.

LCM mice are ex-germ-free mice that were stably associated with the 'Altered Schaedler Flora' (62) comprising approximately 8 species. The representatives with the highest abundance are ASF500 (Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unclassified Lachnospiraceae) and ASF519 (Bacteroidetes; Bacteroidia; Bacteroidales; Porphyromonadaceae; Parabacteroides). Hence those mice harbor a defined, low complexity microbiota (68). Interestingly, this low complexity microbiota does not confer colonization resistance (68).

We used LCM mice to study the role of the microbiota in preventing the rebound of pathogen growth in the gut lumen after the end of the antibiotic therapy. LCM mice (not pretreated with streptomycin) were infected with *S.* Tm^{att}. Antibiotic therapy and experimental readouts were identical to the experimental setup for conventional mice.

We already showed that *S.* Tm^{att} stably colonizes LCM mice over a period of 80 days. Immunized LCM mice resolved gut inflammation at day 40 p.i. and were protected from intestinal

inflammation after re-infection with *S.* Tm^{wt} (22). Thus immunized LCM mice represent the phenotype of asymptomatic excretors. Moreover, those mice allow distinguishing between direct effects of antibiotics (e.g. resolution of inflammation) versus effects of gut microbiota composition for *S.* Typhimurium colonization. For the purpose of our study, those mice provide a highly sensitive system to detect rare events of *S.* Tm re-seeding of the gut lumen from internal reservoirs, such as the MLN, biofilms or intra-epithelial sites in the gall bladder. Each re-seeding event should lead to high level colonization/shedding.

We infected LCM mice orally with $5x10^7$ CFU/g *S.* Tm^{att} and started antibiotic therapy at day 2 p.i.. Mice were treated for 5 days either with ciprofloxacin (15mg/kg; 50 µl by gavage; 2x/d) following the same protocol as for the conventional mice mentioned above.

In line with earlier work, *S.* Tm^{att} efficiently colonized the gut lumen of LCM mice, as indicated by the high levels of *S.* Tm^{att} excretion at day 1 p.i. ($\geq 10^9$ CFU/g feces). Treatment of *S.* Tm^{att} infected LCM with ciprofloxacin resulted in complete pathogen elimination from the feces ($\geq 10^9$ CFU/g at day 1 p.i. and $\leq$ 10 CFU/g feces at day 4 p.i.; $p<0.05$). Re-appearance of fecal *S.* Tm^{att} excretion was observed 6 days after the end of the ciprofloxacin treatment (7/8 mice excreted $\geq 10^5$ CFU/g feces). Those mice kept on excreting *S.* Tm^{att} at high levels ($\geq 10^7$CFU/g feces) until day 40 p.i. (Fig S1B).

We further addressed the question whether this restart of pathogen excretion could be prevented by exposure to a complex microbiota. Therefore, we co-housed ciprofloxacin-treated mice with a healthy SPF C57BL/6 donor mouse after the end of the antibiotic therapy. Carryover of ciprofloxacin from the treated animals to the donor mouse was prevented by keeping the mice on steel-gridded floors during the first days of co-housing. After a first relapse of *S.* Tm^{att} excretion pathogen counts in the feces of these animals dropped to $\leq 10^3$CFU/g feces at day 40 p.i. (Fig S1B, green squares) indicating the *S.* Tm^{att} excretion can be partially suppressed by the exposure to a complex microbiota. The initial relapse might be explained by the kinetics of re-association of LCM mice with a conventional gut flora by animal co-housing (68) and might be decreased by other methods of re-conventionalization, e.g. bacteriotherapy/fecal transplantation.

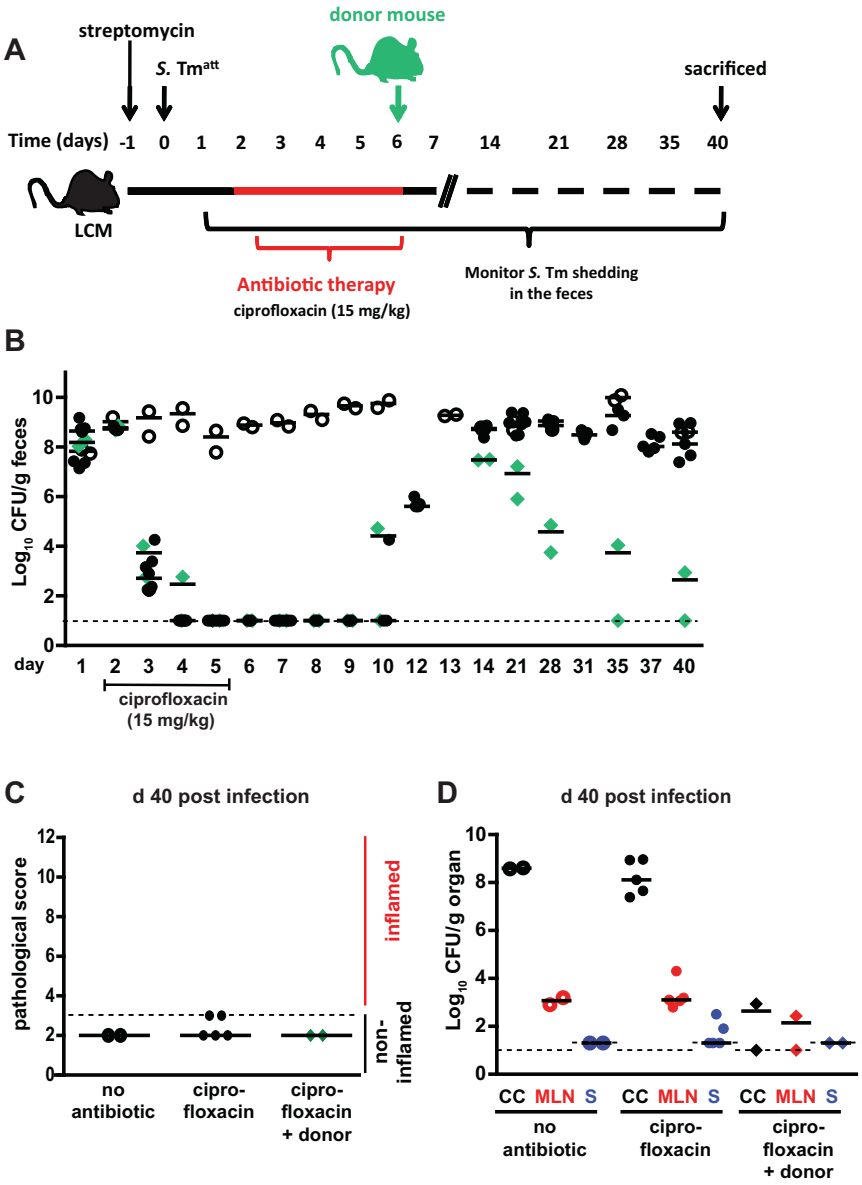
Like conventional mice, LCM mice treated with ciprofloxacin were cured from gut inflammation at day 40 p.i. (Fig. S1C). Ciprofloxacin treatment did not affect *S.* Tm^{att} loads in the MLN at day 40 p.i. (Fig. S1D).

Thus asymptomatic excretors were clearly pronounced in *S.* Tm^{att} infected LCM mice treated with antibiotics. Pathogen re-seeding of the gut lumen was observed for all antibiotic treated LCM mice 6-16 days post antibiotic therapy. Exposure to a complex microbiota did at least partially suppress long term asymptomatic excretion in these mice.

Supplementary Methods

LCM mice had been generated by colonizing ex-germfree C57BL/6 mice with the Altered Schaedler flora (ASF) comprising <20 species (62). Mice, housed in a bubble isolator, were inoculated at eight weeks of age by intra-gastric and intra-rectal administration of 10^7–10^8 CFU of ASF bacteria on consecutive days (www.taconic.com/library). Later, LCM mice (C57BL/6 background) were maintained under barrier conditions in IVCs with autoclaved chow and autoclaved, acidified water. LCM mice were not pretreated with streptomycin but just infected with *S*. Tm^{att}.

Supplementary Figure 1



Supplementary Figure S1:
Ciprofloxacin-treated low complexity flora (LCM) mice show rebound of *S.* Tm^{att} excretion which can partially be suppressed by exposure to a complex microbiota.
A. Experimental scheme to study the role of the gut microbiota on the treatment of acute *Salmonella* diarrhea with ciprofloxacin. **B**. Time course of *S.* Tm^{att} excretion in *S.* Tm^{att} infected LCM mice (black circles) treated for 5 days with ciprofloxacin (15 mg/kg; by gavage, 2x/d). Antibiotic therapy started at day 2 p.i.. Pathogen excretion was monitored over a time period of 40 days. *S.* Tm^{att} excretion is compared to a PBS-treated control group (open circles). A further group of ciprofloxacin-treated mice was co-housed with an SPF C57BL/6 mouse after antibiotic therapy. Pathogen excretion of the antibiotic treated and co-housed animals is depicted in green squares **C.** Mice of the three groups were sacrificed at day 40 p.i. and cecum pathology was analyzed. **D.** *S.* Tm^{att} counts in the cecum content (black), MLN (red) and spleen (blue) at day 40 p.i. of ciprofloxacin treated LCM mice. Dashed lines indicate detection limits.