

Bacterial Colitis Increases Susceptibility to Oral Prion Disease

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Dietary exposure to prion-contaminated materials has caused kuru and variant Creutzfeldt-Jakob disease in humans and transmissible spongiform encephalopathies (TSEs) in cattle, mink, and felines. The epidemiology of dietary prion infections suggests that host genetic modifiers and possibly exogenous cofactors may play a decisive role in determining disease susceptibility. However, few cofactors influencing susceptibility to prion infection have been identified. In the present study, we investigated whether colitis might represent one such cofactor. We report that moderate colitis caused by an attenuated *Salmonella* strain more than doubles the susceptibility of mice to oral prion infection and modestly accelerates the development of disease after prion challenge. The prion protein was up-regulated in intestines and mesenteric lymph nodes of mice with colitis, providing a possible mechanism for the effect of colitis on the pathogenesis of prion disease. Therefore, moderate intestinal inflammation at the time of prion exposure may constitute one of the elusive risk factors underlying the development of TSE.

Prion diseases are fatal neurodegenerative disorders of mammals caused by the conformational conversion of the cellular, glycosylphosphatidylinositol-linked prion protein, known as PrP^C, into a β sheet-rich, aggregated isoform, known as PrP^{Sc} [1]. Prion infections can be induced by oral challenge [2–4] and occur naturally as a result of foodborne contamination, as has been shown for kuru, transmissible mink encephalopathy, bovine spongiform encephalopathy (BSE), and variant Creutzfeldt-Jakob disease (vCJD) [5–8]. vCJD in humans is believed to have been caused by consumption of

beef products contaminated with BSE prions. Despite a probable massive exposure of the European population to the BSE agent, there have been <200 vCJD cases to date [7]. This disproportionately low incidence of infection was very fortunate, yet it was unexpected by most scientists and has led to speculation as to whether specific host risk factors are associated with the development of vCJD [9, 10]. Among such risk factors may be genetically determined, host-encoded modifiers. These include the highly prevalent methionine/valine (Met/Val) polymorphism at codon 129 of the *PRNP* gene [11], given that to date all patients with confirmed vCJD have been found to harbor homozygous Met/Met alleles. However, this observation alone cannot explain the rarity of vCJD, given that the prevalence of Met/Met in humans is >30%.

Nongenetic and extrinsic modifiers may plausibly contribute to susceptibility. One risk factor for prion infection may be an altered immune system. vCJD, chronic wasting disease of cervids, scrapie, and other infectious prions replicate in lymphoid tissues before invading the central nervous system [3, 12–15]. Within lymphoid germinal centers, follicular dendritic cells have been demonstrated to play a key role in prion accumulation [16–18], and their proximity to nerve end-

Received 6 May 2008; accepted 15 August 2008; electronically published 12 December 2008.

Potential conflicts of interest: none reported.

Financial support: Nestlé Research Center; TSEUR Consortium, European Union (A.A.); Swiss National Science Foundation (A.A.); National Competence Centers for Research on Neural Plasticity and Repair (A.A.); US National Institutes of Health (grant K08-AI01802 to C.J.S.); Foundation for Research at the University of Zürich (C.J.S.); US National Prion Research Program (C.J.S. and A.A.); Bonizzi-Theiler Foundation; Max Clöetta Foundation (M.H.).

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The Journal of Infectious Diseases 2009; 199:243–52

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0022-1899/2009/19902-0013\$15.00

DOI: 10.1086/595791

ings influences the kinetics of prion neuroinvasion [19]. Accordingly, modification of the lymphoid system can profoundly influence the pathogenesis of prion disease. For example, mice lacking the complement factor C1q, lymphotoxin- α , lymphotoxin- β , or the lymphotoxin- β receptor either resisted intraperitoneal infection with limiting doses of scrapie prions [20, 21] or experienced a delayed infection [22]. Conversely, recruitment of immune cells caused by chronic inflammation enables prion replication at atypical sites, such as parenchymal organs [23, 24].

After ingestion, the gastrointestinal mucosa affords only limited physical protection against prion infection. Prions have been found to cross mucosal barriers *in vitro* through membranous epithelial cells (M cells), which are specialized sites of antigen sampling for mucosa-associated lymphoid tissue [25]. Several reports have also indicated that prion transport may occur through enterocytes [26, 27] and may be internalized via laminin receptor binding and endocytosis [28]. The number of Peyer patches (PPs) has been shown to influence susceptibility to prion infection, with a decrease in PPs associated with reduced susceptibility in mice exposed orally to scrapie prions [15]. Within 2 weeks of prion ingestion, prions appear to enter peripheral nerves [13] and proceed by invasion of the dorsal motor nucleus of the vagus in the brain, as has been shown in mouse and hamster scrapie studies [29].

Could inflammatory lesions at the mucosal entry site alter susceptibility to prion infection? Inflammatory bowel disease may compromise epithelial tight junctions [30, 31], activate dendritic cells [16], enhance protein antigen uptake [32], and, most crucially, redirect prion replication to the inflamed sites [23, 24]. Hence, intestinal inflammation could conceivably alter the dynamics of prion entry and systemic spread. Gastrointestinal infections caused by viruses, bacteria, and parasites, as well as idiopathic inflammatory diseases, are common in animals and humans, and their contribution to susceptibility to prion infection has not been established.

In the present study, we investigated whether preexisting acute intestinal inflammation alters the susceptibility or kinetics of prion infection. We used a well-established mouse model for *Salmonella enterocolitis* combined with an attenuated *Salmonella enterica* subspecies 1 serovar Typhimurium mutant (M556). *S. Typhimurium* M556 induces a moderate acute suppurative inflammation restricted largely to the cecum and colon within 24 h [33, 34]. Three days after M556 inoculation, we administered scrapie prions by gastric gavage. We found that mice with intestinal inflammation had a significantly higher risk of prion disease.

METHODS

***S. Typhimurium* and prion infections in mice.** Sex- and age-matched specific pathogen-free C57BL/6 mice (groups of 8–12) were maintained under matched specific pathogen-free

conditions. All mouse experiments were approved by the Swiss veterinary authorities. For *S. Typhimurium* infections and controls, mice were transferred into new cages with a metal grid floor, fasted for 4 h, and then treated with 20 mg of streptomycin intragastrically. Twenty hours later, mice were fasted and then orally administered, by gastric gavage, either a mutant strain of *S. Typhimurium* SL1344 (M556; deficient in the TTSS-2 type 3 secretion system; *sseD::aphT* [34]) in 50 μ L containing 4.3×10^7 *S. Typhimurium* organisms or PBS. A third group was not administered streptomycin or *S. Typhimurium*.

Three days later, mice were fasted and orally challenged with 6.4–8.4 log LD₅₀ infectious doses of the RML6 scrapie strain by gastric gavage. A group of control mice was administered *S. Typhimurium* orally, as described above, and then administered uninfected brain homogenate (mock challenge). The cages were changed before each procedure. Fecal samples were collected after infection, homogenized in 500 μ L of PBS, diluted, and plated onto MacConkey agar, and *S. Typhimurium* colonies were counted 24–48 h later.

Mice were weighed, and a blood sample was collected 43 days after scrapie challenge. Two mice in each group were euthanized at 60 and 120 days after scrapie challenge. The intestines were washed in PBS, and separate sections were fixed in formalin, embedded in OCT medium, and snap-frozen in liquid nitrogen for later cryosectioning. Mesenteric lymph nodes (MLNs), spleen, spinal cord, and brain were similarly fixed in formalin and frozen. Tissues from all major organs were fixed in formalin for histological examination. Samples from mice with terminal scrapie were collected similarly.

For the final experiment to measure PrP^C levels, mice were inoculated with *S. Typhimurium* or PBS, as described above. Cecum was embedded in OCT medium and snap-frozen in liquid nitrogen. Cryosections were stained with hematoxylin-eosin (HE). Cecum pathology was evaluated by a pathologist in a blinded manner using a histopathological scoring scheme, as described elsewhere [34, 35].

RNA isolation and real-time polymerase chain reaction (PCR) analysis. Flash-frozen tissues (MLNs) were dissolved in RNA isolation buffer (RLT; Qiagen) and homogenized in a Dispomix device (Medic Tools). RNA was purified as described in the manufacturer's manual (Qiagen). cDNA was generated with a QuantiTect Reverse Transcription Kit, and real-time PCR analysis was performed as described elsewhere, using a Quanti-Fast SYBR Green PCR Kit [23, 24]. The following primer combinations were used for forward (FW) and reverse (RV) primers: *prnp* FW, 5'-GCCAGTGGATCAGTACAGCA-3'; *prnp* RV, 5'-ATCCCACGATCAGGAAGATG-3'.

Statistics. Continuous data are presented as means \pm SDs and were compared using Student's unpaired *t* test. The time to manifestation of terminal scrapie was analyzed within experiments by means of Kaplan-Meier curves. *S. Typhimurium*-infected mice were compared with control mice by the log-rank

test. A stratified Cox regression analysis was performed to compare times to manifestation of scrapie between groups for the pooled data for all 3 experimental doses. Differences were considered statistically significant at $P < .05$ (2-tailed). Statistical analyses were performed using GraphPad Prism (version 4.0) and SPSS (version 13.0) software.

Western blots, histology, immunohistochemistry, and ELISAs.

Western blot, histology, immunohistochemistry, and ELISA methods are included in appendix A, which appears only in the electronic edition of the *Journal*.

RESULTS

S. serovar Typhimurium colitis. We pretreated 2 groups of C57BL/6 wild-type mice with streptomycin, which transiently reduces the density of the commensal gut flora [36] and enables *S. Typhimurium* to colonize the cecum and colon of mice and cause localized inflammation within 3 days [33, 37]. The attenuated *S. Typhimurium* strain M556 triggers acute colitis but lacks a key virulence factor for systemic infection in “susceptible” *Nramp*-negative mouse lines (e.g., C57BL/6). Twenty-four hours later, we gastrically gavaged mice with either PBS or enteropathogenic mutant *S. Typhimurium* deficient in SPI-2 type 3 secretion (12 mice/group). Untreated mice were included as a third group. Three days later, 2 mice per group were euthanized to assess intestinal and systemic pathology, and the remaining mice were exposed to 8.4 log LD₅₀ murine-adapted scrapie brain homogenate (Rocky Mountain Laboratory strain RML6) by gastric gavage. In addition, a control group of 8 mice was pretreated with streptomycin, administered the same *S. Typhimurium* mutant, and gastrically gavaged with normal mouse brain homogenate.

Fecal samples collected during the initial 3 weeks after challenge showed that all *S. Typhimurium*-inoculated groups were colonized. Fecal *S. Typhimurium* counts decreased steadily after the challenge (figure 1A). *S. Typhimurium*-inoculated mice euthanized at the time of exposure to scrapie prions had a moderate colitis characterized by a neutrophil influx into the mucosa and submucosa, crypt abscesses, crypt herniation, lymphocyte-lined lymphatics, submucosal edema, and fibrin exudate on the mucosal surface (compare figure 1B, 1D, and 1E with figure 1C). There were a few additional scattered microabscesses in the liver and spleen. Therefore, the *S. Typhimurium* transiently colonized the intestine and led to inflammation in the cecum and colon, similar to previous findings [36, 38].

To determine whether the lymphoid microarchitecture was disrupted by *S. Typhimurium* infection, we performed an immunohistochemical analysis of the MLNs for B cells, T cells, macrophages, dendritic cells, and follicular dendritic cells (figure 2). We did not detect any gross differences in the composition of the immune cell populations or in the number of germi-

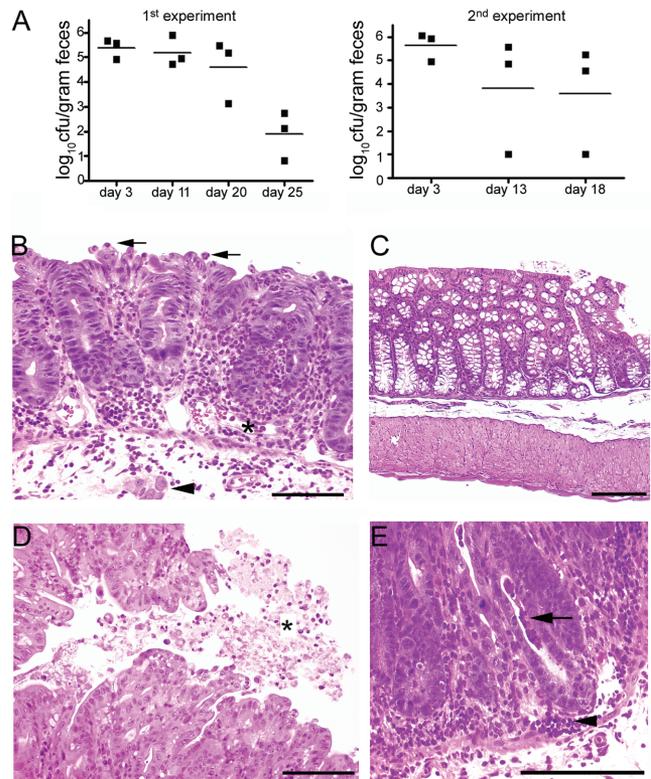


Figure 1. Development of colitis in C57BL/6 mice gavaged intragastrically with *Salmonella* serovar Typhimurium. A, *S. Typhimurium* colonization in feces, assessed as indicated. In 2 independent experiments, the mean concentration of *S. Typhimurium* organisms was rapidly reduced to <2 log colony-forming units per gram of feces by 25 days after infection. B, Colon histopathology at 3 days after *S. Typhimurium* challenge. Results show a widespread influx of neutrophils and some lymphocytes into the lamina propria. Neutrophils were associated with endothelium within vessel lumina (asterisk) and adhered to the mucosal surface (arrows). There was crypt herniation (arrowhead) and a loss of goblet cells. C, Normal colon from a PBS-treated control mouse. D, Fibrin and suppurative exudate on the colonic mucosal surface (asterisk). E, Crypt abscesses (arrow) and pools of inflammatory cells in the mucosa (arrowhead). Scale bars represent 100 μ m.

nal centers. Therefore, inflammatory lesions were largely localized to the cecum and colon.

Mice did not develop diarrhea or other clinical signs of disease but instead grew normally over the subsequent 3 months, with no difference in weight among groups and with normal total blood leukocyte and differential leukocyte counts (figure 3A–3E). Mice had normal serum amyloid A levels at 6 weeks after infection (data not shown). From this we concluded that the *S. Typhimurium* infection was acute, that *S. Typhimurium* was rapidly cleared by 11–25 days after infection, and that mice did not appear to have chronic intestinal inflammatory disease.

In the high-dose (HD) groups exposed to 8.4 log LD₅₀, all mice developed scrapie (table 1). Prion infectivity in the source inoculum was verified by the presence of intraperitoneal and intracerebral control prion infections (table 1).

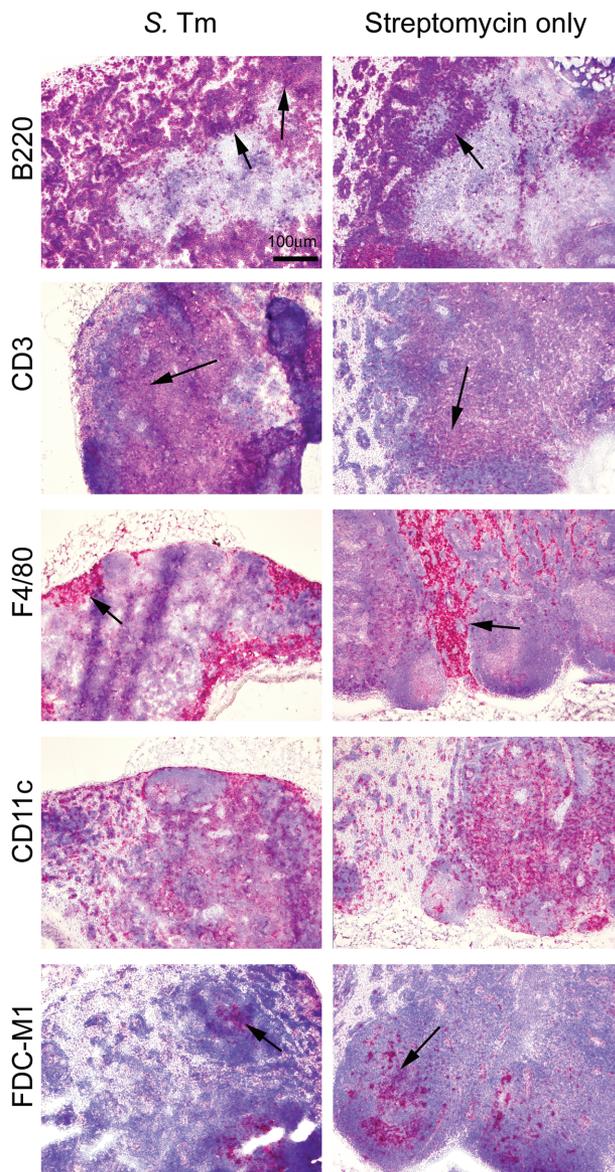


Figure 2. Immunohistochemical stains of mesenteric lymph nodes (MLNs) from mice infected with *Salmonella* serovar Typhimurium. Infection was confirmed by fecal bacterial counts. Immunostains for B and plasmacytoid dendritic cells (B220) and T cells (CD3) showed that B and T cells were distributed in cortical (arrows) and paracortical (arrows) areas, respectively, in both the *S. Typhimurium*-infected and non-*S. Typhimurium*-infected mice. Macrophages (F4/80) (arrows) and dendritic cells (CD11c) were primarily localized to the subcapsular sinus and medullary cords, and follicular dendritic cells (FDC-M1) were present in the follicular germinal centers in the cortex (arrows), indicating that the overall histological state of the MLNs was not dramatically affected by *S. Typhimurium* infection. *S. Tm*, *S. Typhimurium*.

Increase in susceptibility to prions due to concurrent infection. We suspected that subtle differences in the kinetics of prion infection may be masked by a high, saturating dose of prions. Therefore, the experiment was repeated with 7.4 and 6.4 log LD₅₀ of RML6 prions (medium-dose [MD] and low-dose [LD] groups, respectively). We then determined whether, at

these lower doses, intestinal inflammation had an influence on the number of mice with terminal prion disease, as assessed by clinical signs and the detectability of PrP^{Sc} in the brain by Western blot (attack rate). We found that the attack rate of scrapie was influenced by coinfection with *S. Typhimurium*. We pooled all control mice without *S. Typhimurium* infection for the statistical analyses. This procedure enabled us to formally assess the effect of *S. Typhimurium* coinfection on the pathogenesis of prion infection. Because of the small number of animals in the subgroups, however, this comparison did not reveal any potential effects of isolated streptomycin treatment. In the MD group, scrapie developed in 4 (67%) of 6 mice with *S. Typhimurium* coinfection versus only 5 (45%) of 11 without coinfection. The comparable numbers in the LD group were 1 (17%) of 6 versus 1 (9%) of 11 (table 1).

We then performed a survival analysis of the time from the inoculation with prions to the manifestation of terminal scrapie signs (survival time) (figure 4). In the HD groups, the median \pm SE survival time was 224 \pm 4 days for mice with and 230 \pm 0.5 days for mice without *S. Typhimurium* infection. By 275 days after inoculation, no mice were free of scrapie. By comparison, in the MD groups, the median \pm SE survival time was 216 \pm 5 days for mice infected with *S. Typhimurium*, whereas so few mice developed disease that the median was not reached for mice without *S. Typhimurium* coinfection. By 275 days after inoculation, only 33% of *S. Typhimurium*-infected mice were free of scrapie, with no PrP^{Sc} detectable in the brain by Western blot or immunohistochemistry, but 55% of mice without *S. Typhimurium* coinfection were free of scrapie. At the lowest prion dose, 83% and 91% were free of scrapie in the *S. Typhimurium*-infected and non-*S. Typhimurium*-infected groups, respectively.

We next compared the attack rate in the HD, MD, and LD groups by stratified Cox regression. We found a significant difference in the risk of terminal prion disease associated with *S. Typhimurium* coinfection ($P = .037$). The hazard for contracting scrapie was increased by 2.3-fold with *S. Typhimurium* infection (95% confidence interval, 1.1–5.1-fold).

Modest acceleration of the pathogenesis of scrapie due to colitis. To determine whether the kinetics of prion infection differed depending on intestinal inflammation, we euthanized mice at 2 time points preceding the development of clinical disease: 60 and 120 days after inoculation. We compared the accumulation of prions in the MLNs and spleen among the HD groups. PrP^{Sc} was detected by histoblot analysis in MLNs of all scrapie-challenged mice at 60 days after inoculation (figure 5A), a finding in accordance with the 100% attack rate in the HD group.

However, the kinetics of prion disease varied among the MD groups. By 60 days after inoculation, PrP^{Sc} was detected in MLNs by Western blot in both of 2 mice exposed to *S. Typhimurium* and in 2 of 4 mice exposed only to prions, suggesting accelerated

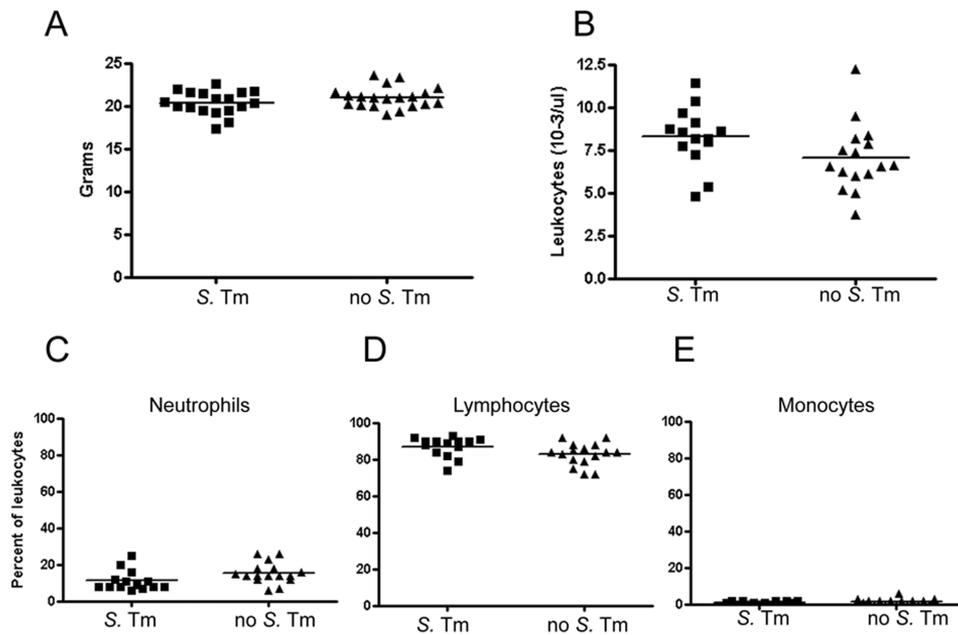


Figure 3. Normal body weight and unchanged complete blood counts in mice infected with *Salmonella* serovar Typhimurium M556. *A*, Body weight of *S. Typhimurium*-infected and control mice 43 days after prion exposure ($P = .14$, Student's unpaired 2-tailed *t* test). *B*, Total leukocyte counts. Counts showed no significant difference between the *S. Typhimurium*-infected and control mice ($P = .08$). *C–E*, Differential counts of neutrophils, lymphocytes, and monocytes. These counts also showed no or slightly significant differences between *S. Typhimurium*-infected and control mice ($P = .04$, $P = .06$, and $P = .26$, respectively). *S. Tm*, *S. Typhimurium*.

prion spread due to mucosal inflammation (figure 5*B*). In the spleen, both of 2 *S. Typhimurium*-coexposed mice had detectable PrP^{Sc}, compared with only 1 of 4 mice without *S. Typhimurium* infection (figure 5*C*). We quantified the intensity of the Western blot signals among the MLNs and spleens and found significantly higher PrP^{Sc} levels in mice with *S. Typhimurium* coinfection ($P = .02$ and $P = .006$, respectively, unpaired Student's *t* test). Histoblots of MLNs and spleen showed the same results (figure 5*C* and 5*D*). By 120 days after inoculation, nearly all mice had accumulated PrP^{Sc} in the MLNs and spleen, except for 1 mouse that was administered prions only (figure 5*B* and 5*C*). Overall, these results suggest a trend toward accelerated prion disease associated with concomitant colitis.

For the LD scrapie groups, PrP^{Sc} was detected in the MLNs in 1 of 2 mice coexposed to *S. Typhimurium* at 60 days after inoc-

ulation. None of the other mice receiving a low dose of prions had detectable PrP^{Sc} in the MLNs (0/4 mice; data not shown). The *S. Typhimurium*-exposed mice in the MD and LD groups consistently showed detectable PrP^{Sc} in the MLNs and spleen more frequently than non-*S. Typhimurium*-exposed mice. It is possible that mice without detectable PrP^{Sc} in the MLNs or spleen would never have developed scrapie, and the *S. Typhimurium*-induced inflammation may have led either to accelerated scrapie or to an increase in susceptibility to scrapie at these limiting doses of prions.

Increase in PrP^C expression in cecum and MLNs due to *Salmonella* infection. The accelerated kinetics associated with coinfection may have been due to increased PrP^C expression caused by inflammation. To determine whether the *S. Typhimurium* infection led to PrP^C up-regulation, we gavaged groups

Table 1. Incubation periods in RML6 prion-infected mice at 3 doses.

Colon condition	Oral infection					
	8.4 log LD ₅₀		7.4 log LD ₅₀		6.4 log LD ₅₀	
	Attack rate ^a	Incubation period, days ^b	Attack rate ^a	Incubation period, days ^b	Attack rate ^a	Incubation period, days ^b
Inflammation	6/6 (100)	225 ± 2	4/6 (67)	215 ± 3	1/6 (17)	213 (>560 for 5 mice)
No inflammation	12/12 (100)	232 ± 2	5/11 (45) ^c	236 ± 12	1/11 (9) ^c	218 (>560 for 10 mice)

^a Data are no. of mice with terminal scrapie/total no. of mice (%).

^b Data are the no. of days between prion inoculation and the development of clinical signs of terminal scrapie, given as means ± SE for diseased animals (except for the lowest dose, because only 1 mouse was diseased for each condition).

^c One mouse in each group died of causes unrelated to scrapie and was not included in the calculation of the incubation period.

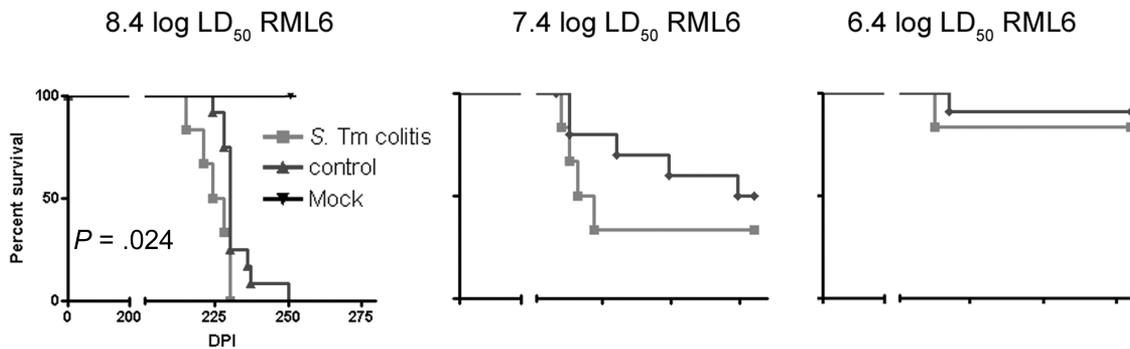


Figure 4. Survival curves for mice infected with *Salmonella enterica* serovar Typhimurium and control mice challenged with 3 doses of prions. Only mice receiving the highest dose of prions showed an attack rate of 100%. The incubation period from prion challenge to the development of terminal scrapie was significantly shorter in *S. Typhimurium*-infected mice than in control mice. DPI, days post inoculation; *S. Tm*, *S. Typhimurium*.

of 6 C57BL/6 mice with either *S. Typhimurium* or PBS, quantified the *S. Typhimurium* colonization, confirmed the colitis with histological sections of intestine (figure 6A–6C), and measured PrP^C expression in the cecum, MLNs, and spleen by ELISA. We found that PrP^C levels were significantly increased by the *S. Typhimurium* infection in the cecum (3-fold; $P < .001$, unpaired Student's *t* test) and MLNs (1.5-fold; $P = .013$), but not in the spleen (figure 6D). Among the control mice, PrP^C expression levels were ~10-fold higher in the MLNs than in the spleen. Prion mRNA levels were assessed for MLNs and were also found to be significantly elevated in *S. Typhimurium*-infected mice, compared with those in uninfected mice (~2-fold; $P = .007$) (figure 6E). Thus, acute mucosal inflammation increases the PrP^C concentration in the affected tissue. This may translate into an elevated susceptibility to prion infection.

DISCUSSION

We found that moderate acute intestinal inflammation leads to an enhanced risk of contracting prion disease on oral exposure. After exposure to limiting dilutions of prions, the risk of prion infection more than doubled with concurrent intestinal inflammation. This increase in susceptibility to prion infection associated with colitis was accompanied by only a slight acceleration in the kinetics of infection. The *S. Typhimurium* infection caused an acute yet relatively mild inflammation localized to the distal gastrointestinal tract, with no visible clinical signs of disease in the mice. This model is physiologically similar to mild forms of acute inflammatory colitis in humans and other animals [39]. Therefore, these results suggest that colitis at the time of exposure to prions may be a realistic exogenous risk factor for prion infection.

Several mechanisms may plausibly underlie the enhanced susceptibility to prion infection. In the *S. Typhimurium*-infected mice, PrP^C expression was increased 3-fold in the cecum and ~1.5-fold in the draining MLNs, consistent with increased PrP^C expression detected in the stomachs of humans with

Helicobacter-induced gastritis [40]. Because PrP^C expression directly correlates with incubation period (or the time from prion inoculation to development of terminal scrapie [41]), the elevated PrP^C expression in the cecum may lead to increased prion conversion and accelerated prion transport and neuroinvasion. A second possible mechanism is that prion uptake is increased through a disrupted epithelial barrier, which has been shown to enhance protein uptake in Crohn disease [32]. This would effectively increase the systemic dose of prions. A third mechanism may involve increased trafficking of prions to draining lymphoid follicles in migrating *S. Typhimurium*-containing dendritic cells [42–44].

The finding of an eroded mucosa associated with an enhanced risk for prion disease is consistent with other reports. Laceration of the tongue had a marked effect on susceptibility to lingually applied prions [45], with the incidence of prion disease reaching 100% versus 29% in mice with intact tongues. Another study found that scarification of the oral cavity followed by prion exposure showed a similar trend, with 100% of scarified mice versus 71% of nonscarified mice becoming infected with prions [46]. These results might suggest that the enhanced susceptibility to prion disease in all 3 models is due to a breach in the mucosal barrier or enhanced epithelial permeability, allowing increased prion uptake.

Prion infection after oral exposure requires PPs in mice, and any numerical decrease in PPs reduces the risk of prion infection [15]. However, a recent study found that reducing the number of PPs had no effect on scrapie infection, provided that numerous isolated lymphoid follicles containing follicular dendritic cells were present [47]. Another study examined the correlation between the PP surface area, follicle density, PP weight, or number of PPs and the risk of prion infection in sheep, cattle, and humans. A significant correlation was found in all 3 prion host species, suggesting a link between PP development and natural susceptibility to transmissible spongiform encephalopathy [48]. In our experiments, acute *S. Typhimurium* infection did not

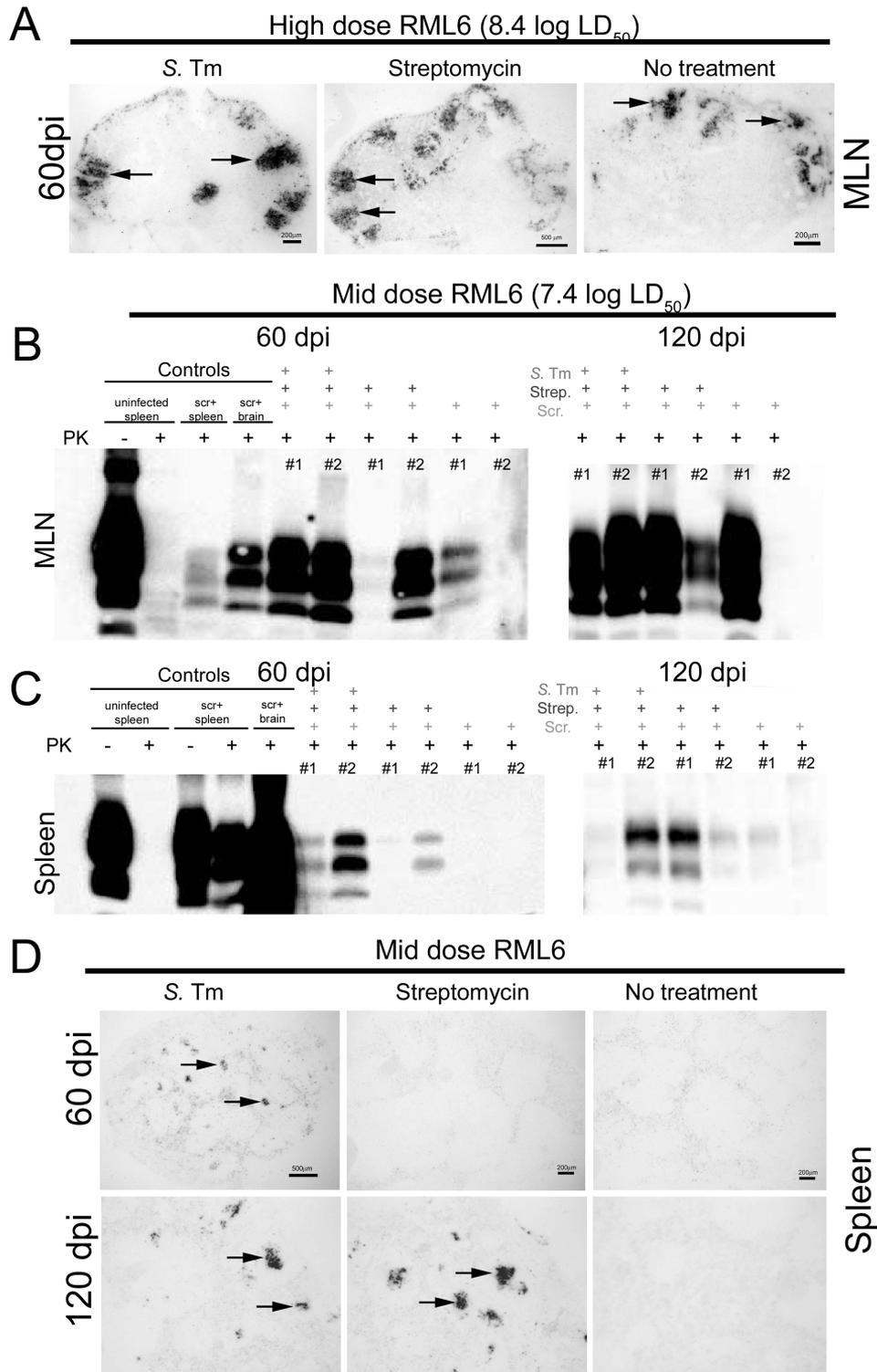


Figure 5. PrP^{Sc} in mesenteric lymph nodes (MLNs) and spleens of mice during early prion infection. *A*, Histoblots from mice infected with *Salmonella enterica* serovar Typhimurium and from control mice. All histoblots showed PrP^{Sc} in MLNs by 60 days after inoculation (arrows), indicating that no difference was seen in MLNs at this time point with the highest prion dose. *B*, Western blot analysis of MLNs from mice infected with a limiting dose of prions at 60 and 120 days after inoculation. Blots indicated that some mice had no detectable PrP^{Sc}, probably owing to either slow or absent prion infection. PK, proteinase K treatment of tissue homogenates. *C*, Splenic PrP^{Sc} levels from the same mice as in panel *B*. Levels were less than those for MLNs at the same time points. *D*, Histoblots illustrating black PrP^{Sc} deposition in the spleen from the *S. Typhimurium*-infected mouse at 60 days after inoculation (arrows) but not in the 2 non-*S. Typhimurium*-infected mice as well as in the *S. Typhimurium*-infected and streptomycin-treated mouse (arrows) but not in the second non-*S. Typhimurium*-infected mouse at 120 days after inoculation. These findings are indicative of either delayed or absent prion infection. dpi, days post inoculation; *S. Tm*, *S. Typhimurium*.

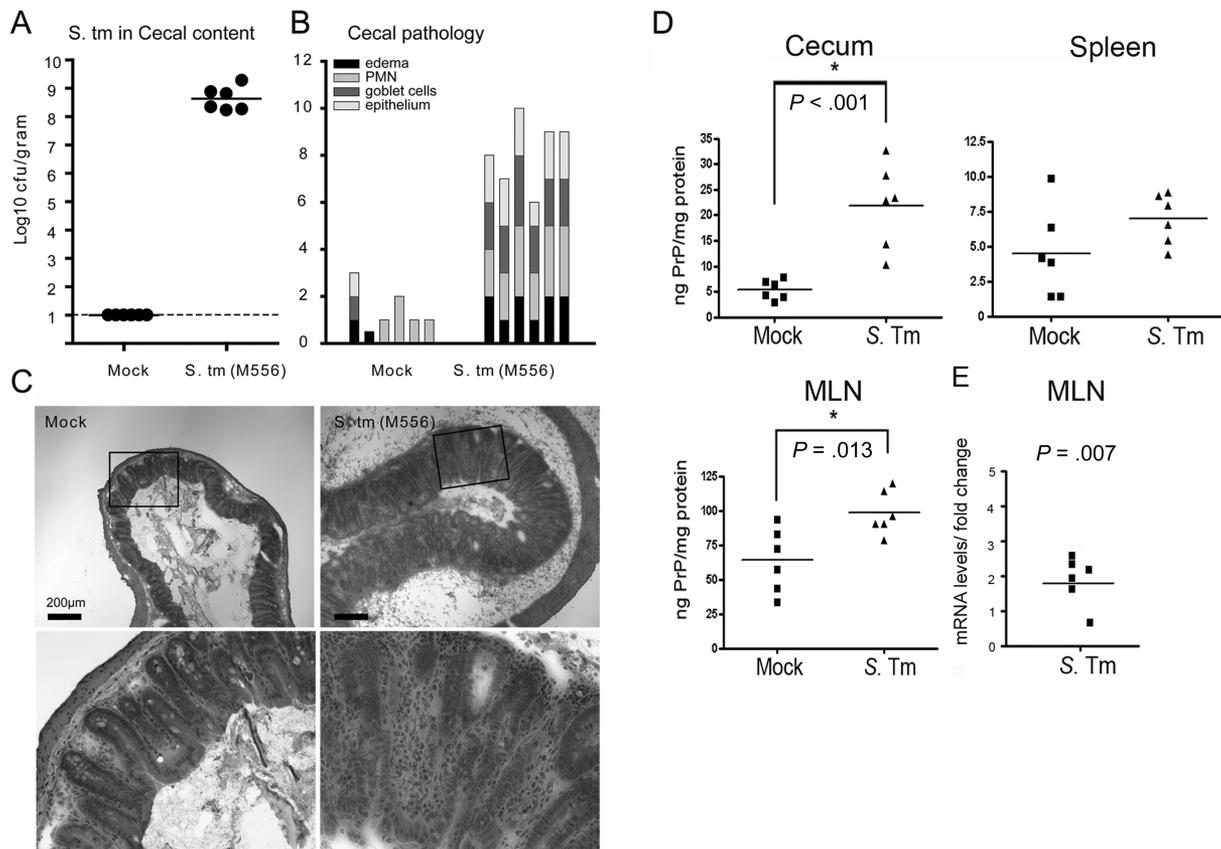


Figure 6. Streptomycin-pretreated wild-type C57BL/6 mice 3 days after challenge with *Salmonella enterica* serovar Typhimurium M556 (*sseD::aphT*; 5×10^7 cfu administered intragastrically [34]) ($n = 6$) or after mock challenge with PBS ($n = 6$). *A*, Cecal *S. Typhimurium* loads. Mice were euthanized, and cecal *S. Typhimurium* loads were determined by plating on MacConkey agar containing 50 $\mu\text{g/mL}$ streptomycin. The dotted line indicates the limit of detection, and the black bar indicates the median. *B*, Cecal pathology. Cecal tissues were embedded, and inflammation (or lack thereof) was confirmed by histopathological evaluation of hematoxylin-eosin–stained tissue sections; tissues were scored with respect to submucosal edema, neutrophil infiltration, loss of goblet cells, and epithelial disruption. *C*, Representative images. *Left*, mock infected; *right*, *S. Typhimurium* infected. Scale bars represent 200 μm . Lower images show enlargements of areas indicated by boxes in the upper images. *D*, PrP^C ELISA measurements in the cecum, mesenteric lymph nodes (MLNs), and spleen from mice infected with *S. Typhimurium* and control mice. PrP^C levels were elevated in the cecum and MLNs but not in the spleen, owing to the SPI-2 mutant *S. Typhimurium* infection. *E*, Levels of *prnp* mRNA in MLNs. Levels were elevated 2-fold in *S. Typhimurium*–infected mice, compared with those in control mice. S. Tm, *S. Typhimurium*.

increase the size or number of PPs, suggesting that the latter are not limiting or do not contribute to the increased risk of prion disease with *S. Typhimurium* infection.

Previous studies have shown that lymphofollicular inflammatory foci in nonlymphoid organs, including the liver, kidney, mammary gland, and pancreas, can accumulate PrP^{Sc} [23, 49]. However, in contrast to the results shown here, the inflammation did not affect the susceptibility to or the kinetics of scrapie prion infection [23]. These differences are not surprising, considering that, in the present study, inflammation occurred at a mucosal surface, where prion uptake would take place. An array of very common inflammatory lesions may therefore alter the quantity of prions crossing the mucosal barrier, including buccal erosions, glossitis, erosive tonsillitis, gastric ulcers, and enteritis. All of these conditions, therefore, are likely to increase the likelihood of infection on exposure to prions.

Extensive epidemiologic investigations of vCJD cases have been performed in the United Kingdom to identify risk factors for the development of disease, including interviews with patients' relatives and reviews of dental records [9, 10]. As might be expected, frequent consumption of beef products likely to contain mechanically recovered meat or head meat, which may have been contaminated with central nervous tissue, has been associated with an increased risk of vCJD [9]. Other than the latter, few exogenous risk factors have correlated with the development of vCJD. On the other hand, it may be challenging to recognize any risk posed by small lesions along the gastrointestinal tract, because (1) lesions may have occurred years before the onset of prion disease, (2) mild gastrointestinal disease is extremely common, and (3) many cases are not treated by physicians and, therefore, would not be reported. However, considering the results of the present study as well as the frequency of gastrointes-

tinal tract erosions and ulcerations caused by infectious and noninfectious diseases, gastrointestinal tract pathology may plausibly exert a marked effect on susceptibility to prion disease in humans and animals.

Acknowledgments

We thank Marianne König and the histopathology and animal care staff at the University of Zurich for technical support.

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