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Bacterial Colitis Increases Susceptibility to Oral Prion Disease

Christina J. Sigurdson[1,2], **Mathias Heikenwalder**[1], **Giuseppe Manco**[1], **Manja Barthel**[3], **Petra Schwarz**[1], **Bärbel Stecher**[3], **Nike J. Krautler**[1], **Wolf-Dietrich Hardt**[3], **Burkhardt Seifert**[4], **Andrew J. S. MacPherson**[5,^], **Irène Corthesy**[6], and **Adriano Aguzzi**[1,*]

[1]Institute of Neuropathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH 8091 Zurich, Switzerland [2]Department of Pathology, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0612, USA [3]Institute of Microbiology, ETH, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland [4]Biostatistics Unit, ISPM, University of Zurich, Zurich, Switzerland [5]Institute of Experimental Immunology, University Hospital of Zurich, Schmelzbergstrasse 12, CH 8091 Zürich, Switzerland [6]Nestlé Research Center, Nestec Ltd., CH-1000 Lausanne 26, Switzerland

Abstract

Dietary exposure to prion-contaminated materials has caused kuru and variant Creutzfeldt-Jakob disease in humans, and transmissible spongiform encephalopathies (TSEs) of cattle, mink, and felines. The epidemiology of dietary prion infections suggest that host genetic modifiers, and possibly exogenous cofactors, may play a decisive role in determining disease susceptibility. However, few cofactors influencing prion susceptibility have been identified. Here we investigated whether colitis might represent one such cofactor. We report that moderate colitis caused by an attenuated strain of Salmonella 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 upregulated in intestines and mesenteric lymph nodes of mice with colitis, providing a possible mechanism for the impact of colitis onto prion pathogenesis. Therefore, moderate intestinal inflammation at the time of prion exposure may constitute one of the elusive risk factors underlying the development of TSE.

Keywords

Prion; TSE; Oral infection; Susceptibility; Salmonella; Neurodegeneration; Colitis

Introduction

Prion diseases are fatal neurodegenerative disorders of mammals caused by the conformational conversion of the cellular, GPI-linked prion protein, 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 food-borne contamination, as shown for kuru, transmissible mink encephalopathy (TME), 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 fewer than 200 vCJD cases to date

*Corresponding author: Adriano Aguzzi, Institute of Neuropathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH–8091 Zürich, Switzerland, Tel.: +41 (1) 255 2107, Telefax: +41 (1) 255 4402, adriano.aguzzi@usz.ch.
^Current address: Department of Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada

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[7]. This disproportionately low incidence of infection was very fortunate, yet it was unexpected by most scientists, and has led to speculation of specific risk factors associated with the vCJD-infected individuals [9,10]. Among such risk factors there 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], since all confirmed vCJD patients to date have been found to encode homozygous Met/Met alleles. However, this observation alone cannot explain the rarity of vCJD, since the prevalence of Met/Met in humans is >30%.

Non-genetic 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 (CWD), scrapie, and other prion diseases replicate in the lymphoid tissues prior to invading the central nervous system (CNS) [3,12-15]. Within lymphoid germinal centers, follicular dendritic cells (FDCs) were demonstrated to play a key role in prion accumulation [16-18], and their proximity to nerve endings influences the kinetics of prion neuroinvasion [19]. Accordingly, modifying the lymphoid system can profoundly influence prion pathogenesis. For example, mice lacking complement factor C1q, or lymphotoxin-α, lymphotoxin-β, or lymphotoxin-β receptor resist intraperitoneal infection with limiting doses of scrapie [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 were found to cross mucosal barriers in vitro through membranous epithelial cells (M cells), which are specialized sites of antigen sampling for mucosal associated lymphoid tissue (MALT) [25]. Several reports also indicate that prion transport may occur through enterocytes [26,27], and may be internalized via laminin receptor binding and endocytosis [28]. The number of Peyer's patches (PP) has been shown to influence prion susceptibility, with a decrease in PP associated with reduced susceptibility in mice exposed orally to scrapie [15]. Within two 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 shown in mouse and hamster scrapie studies [29].

Could inflammatory lesions at the mucosal entry site alter prion susceptibility? 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 prion susceptibility has not been established.

Here 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 (*S.* Tm) (M556). *S.* Tm M556 induces a moderate, acute suppurative inflammation restricted largely to the caecum and colon within 24 hours [33,34]. Three days later we administered scrapie prions by gastric gavage. We found that mice suffering from intestinal inflammation were at significantly higher risk for prion disease.

Methods

S. *Tm* and prion infections in mice

Sex- and age-matched specified pathogen-free (SPF) C57BL/6 mice (groups of n=8-12) were maintained under SPF conditions. All mouse experiments were approved by the Swiss Veterinary Authorities. For *S.* Tm infections and controls, mice were transferred into new cages with a metal grid floor, fasted for four hours and then treated with 20mg streptomycin intragastrically (i.g.). Twenty hours later, mice were fasted and then orally dosed by gastric gavage with a mutant strain of *S.* Tm SL1344 (M556; deficient in the TTSS-2 type three secretion system; *sseD::aphT* [34]), 50ul containing 4.3×10^7 *S.* Tm organisms, or with phosphate buffered saline (PBS). A third group was not dosed with streptomycin or *S.* Tm.

Three days subsequent, mice were fasted and orally challenged with 6.4-8.4 log LD_{50} infectious doses of the RML6 scrapie strain by gastric gavage. A group of control mice was dosed with *S.* Tm as described above, and then dosed with uninfected brain homogenate orally (mock). The cages were changed prior to each procedure. Fecal samples were collected post-infection, homogenized in 500μl PBS, diluted, plated onto MacConkey agar, and *S.* Tm colonies were counted 24-48 hours later.

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

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

RNA isolation and Real-time PCR analysis

Flash frozen tissues (MLN) were dissolved in RNA isolation buffer (RLT; Qiagen) and homogenized in a Dispomix (Medic Tools™). RNA was purified as described by the manufacturer manual (Qiagen). cDNA generation was performed with a QuantiTect®, Reverse Transcription kit and Real-time PCR analysis was performed as recently described using a QuantiFast™ SYBR®Green PCR kit [23,24]. The following primer combinations were used [forward primer (FW), reverse primer (RV)]: *prnp FW:* 5'-GCCAGTGGATCAGTACAGCA-3'. *prnp RV:* 5'-ATCCCACGATCAGGAAGATG-3'.

Statistics

Continuous data are presented as mean ± standard deviation and compared using Student's unpaired t-test. Time to manifestation of terminal scrapie was analysed within experiments using Kaplan-Meier curves. *S.* Tm-infected mice were compared to control mice using the log-rank test. A stratified Cox-regression was performed to compare time to manifestation of scrapie between groups for the pooled data of all three experimental doses. Two-tailed p-values < 0.05 are considered statistically significant. Statistical analyses were perfomed using GraphPad Prism™ and SPSS 13.0.

Western blots, histology, immunohistochemistry, and ELISA methods are included in the Supplementary Online document.

Results

Salmonella enterica serovar typhimurium colitis

We pretreated two groups of C57BL/6 wild type (WT) mice with streptomycin, which transiently reduced the density of the commensal gut flora [36] and enables *S.* Tm to colonize the caecum and colon of mice and cause localized inflammation within 3 days [33,37]. The attenuated *S.* Tm strain M556 triggers acute colitis but lacks a key virulence factor for systemic infection in "susceptible" Nramp-negative mouse lines (i.e. C57BL/6). Twenty-four hours later we gastrically gavaged mice with (i) enteropathogenic mutant *S.* Tm, deficient in SPI-2 type three secretion or (ii) PBS (n=12 mice/group). Untreated mice were included as a third group. Three days later, two mice per group were sacrificed to assess intestinal and systemic pathology, and the remaining mice were exposed to 8.4 log LD_{50} murine-adapted scrapie brain homogenate (Rocky Mountain Laboratory strain, RML6) by gastric gavage. In addition, a control group of eight mice was pretreated with streptomycin, dosed with the same mutant *S.* Tm, and gastrically gavaged with normal mouse brain homogenate.

Fecal samples collected during the initial three weeks post-challenge showed that all *S.* Tm-inoculated groups were colonized. Fecal *S.* Tm counts decreased steadily post-challenge (figure 1A). *S.* Tm-treated mice sacrificed at the time of exposure to scrapie 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 (figure 1, compare B,D,E with C). There were a few additional scattered microabscesses in the liver and spleen. Therefore the *S.* Tm transiently colonized the intestine and led to inflammation in the caecum and colon, similar to previously published reports [36, 38].

To determine whether the lymphoid microarchitecture was disrupted by the *S.* Tm infection, we performed an immunohistochemical analysis on the mesenteric lymph node (MLN) 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 germinal centers. Therefore, inflammatory lesions were largely localized to the caecum and colon.

Mice did not develop diarrhea or other clinical signs of disease, but instead grew normally over the subsequent three months with no difference in weight among groups, and with normal total blood leukocyte and differential leukocyte cell counts (figure 3A-E). Mice had normal serum amyloid A levels at 6 weeks post-infection (data not shown). From this we concluded that the *S.* Tm infection was acute, *S.* Tm was rapidly cleared by 11-20 days post-infection, and mice did not appear to suffer from chronic intestinal inflammatory disease.

For the high dose (HD) groups exposed to 8.4 log LD_{50}, all mice developed scrapie (table 1). Intraperitoneal and intracerebral control prion infections verified prion infectivity in the source inoculum (table 1).

Prion susceptibility increases with concurrent infection

We suspected that subtle differences in the kinetics of prion infection may be masked by a saturating, high dose of prions. Therefore the experiment was repeated with 7.4 log LD_{50} and 6.4 log LD_{50} of RML6 prions (referred to as mid- and low-dose group, MD and LD, respectively). We then determined whether the intestinal inflammation had an influence on the number of mice developing terminal prion disease, as assessed by clinical signs and detectable

PrP^{Sc} in brain by western blots (attack rate), at these lower doses of prions. Here we found the attack rate of scrapie was influenced by co-infection with *S.* Tm. We pooled all control mice without *S.* Tm infection for the statistical analyses. This procedure enabled us to formally assess of the effect of *S.* Tm co-infection on prion pathogenesis but, because of the small number of animals in subgroups, did not reveal any potential effects of isolated streptomycin treatment. Whereas 4 of 6 mice (67%) developed scrapie with *S.* Tm co-infection from the MD group, only 5 of 11 (45%) mice without *S.* Tm infection developed scrapie. Within the LD group, 1 of 6 *S.* Tm-infected mice (17%) versus 1 of 11 mice without *S.* Tm co-infection (9%) developed scrapie (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). For the HD groups, the median survival time ± standard error (M ± SE) for survival was 224 ± 4 days with *S.* Tm infection and 230 ± 0.5 days in mice without *S.* Tm infection. By 275 days post-inoculation (dpi), no mice were free of scrapie. By comparison, for the MD groups, the M ± SE was 216 ± 5 days for mice infected with *S.* Tm, whereas the median was not reached for mice without *S.* Tm co-infection. By 275 dpi, only 33% of *S.* Tm-infected mice were free from scrapie, with no detectable PrP^{Sc} by western blot or by immunohistochemistry in brain, whereas 55% were scrapie-free without *S.* Tm co-infection. At the lowest prion dose, 83% or 91% were scrapie-free in the *S.* Tm or non *S.* Tm-infected groups, respectively.

We next compared the attack rate in the HD, MD, and LD groups by stratified Cox regression. Here we found a significant difference in the risk of terminal prion disease associated with the *S.* Tm co-infection (p=0.037). The hazard for contracting scrapie was increased by 2.3-fold with a *S.* Tm infection (95% confidence interval: 1.1 - 5.1).

The pathogenesis of scrapie infection is modestly accelerated by colitis

To determine whether the kinetics of prion infection differed due to intestinal inflammation, we sacrificed mice at two time points preceding the development of clinical disease: 60 and 120 days post inoculation (dpi). We compared the accumulation of prions in the mesenteric lymph nodes (MLN) and spleen among the high-dose (HD) groups. PrP^{Sc} was detected in MLNs of all scrapie-challenged mice at 60 dpi by histoblot analysis (figure 5A), which is in accordance with the 100% attack rate in the HD group. However, the kinetics of prion disease varied among the MD groups. By 60dpi, PrP^{Sc} was detected by western blot analysis in the MLN in 2 of 2 mice exposed to *S.* Tm, and in 2 of 4 mice exposed only to prions, suggestive of accelerated prion spread due to mucosal inflammation (figure 5B). In the spleen, 2 of 2 *S.* Tm co-exposed mice had detectable PrP^{Sc}, whereas only 1 of 4 mice without *S.* Tm had detectable PrP^{Sc} (figure 5C). We quantified the intensity of the western blot signals among the MLN and spleens and found significantly higher PrP^{Sc} levels due to the *S.* Tm co-infection (unpaired Student's t test, p=.02 and p=.006, respectively). Histoblots of MLN and spleen showed the same results (figure 5C-D). By 120 dpi, nearly all mice had accumulated PrP^{Sc} in the MLN and spleen, with the exception of 1 mouse that was dosed with prions only (figure 5B-C). Overall, these results suggest a trend towards accelerated prion disease associated with concomitant colitis.

For the LD scrapie groups, PrP^{Sc} was detected in the MLN in 1 of 2 mice co-exposed to *S.* Tm at 60 dpi. All other mice receiving a LD of prions had no detectable PrP^{Sc} in the MLN (0/4 mice; data not shown). The *S.* Tm-exposed mice in the MD and LD groups consistently showed detectable PrP^{Sc} in MLN and spleen in more mice compared to non *S.* Tm- exposed mice. It is possible that mice showing no detectable PrP^{Sc} in MLN or spleen would have never developed scrapie. Therefore, the *S.* Tm-induced inflammation may have led to either accelerated scrapie or to an increase in scrapie susceptibility at these limiting doses of prions.

Salmonella infection causes an increase in PrP^C expression in the caecum and MLN

Accelerated kinetics associated with a co-infection may have been due to increased PrP^C expression caused by inflammation. To determine whether the *S.* Tm infection led to PrP^C upregulation, we gavaged groups of six C57BL/6 mice with either *S.* Tm or PBS, quantified the *S.* Tm colonization, confirmed the colitis with histologic sections of intestine (figure 6A-C), and measured PrP^C expression in caecum, MLN, and spleen by PrP ELISA assay. We found that PrP^C levels were significantly increased by the *S.* Tm infection in caecum (3-fold, unpaired Student's t test, p=0.0008) and MLN (1.5 fold, p=0.0134), but not in spleen (figure 6D). Within the control mice, the PrP^C expression levels were approximately 10-fold higher in MLN compared to spleen. PrP mRNA levels were assessed for MLN and were also found to be significantly elevated in *S.* Tm-infected mice compared to uninfected mice (approximately 2-fold, p=0.007, 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 developing prion disease upon oral exposure. When exposed to limiting dilutions of prions, the risk of contracting a prion infection more than doubled with concurrent intestinal inflammation. This increase in prion susceptibility associated with colitis was accompanied by only a slight acceleration in the kinetics of infection. The *S.* Tm 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 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 prions. In the *S.* Tm infected mice, PrP^C expression was increased by 3-fold in the caecum and increased by approximately 1.5-fold in the draining MLN, consistent with increased PrP^C expression detected in the stomach of humans with *Helicobacter*-induced gastritis [40]. Since 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 caecum may lead to increased PrP 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's disease [32]. This would effectively increase the systemic dose of prions. A third mechanism may be due to an increase in trafficking of prions to draining lymphoid follicles in migrating, *S.* Tm-containing dendritic cells (DCs) [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 tongue. 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 prion-infected [46]. These results might suggest that the enhanced susceptibility to prion disease in all three models may be due to a breach in the mucosal barrier or enhanced epithelial permeability, and increased prion uptake.

Prion infection after an oral exposure requires Peyer's patches (PP) in mice, and any numerical decrease of PP reduces the risk of prion infection [15]. However, a recent study found that reducing the number of Peyer's patches had no effect on the scrapie infection, provided that numerous isolated lymphoid follicles containing FDCs were present [47]. Another study

examined the correlation between the PP surface area, follicle density, PP weight, or number of PP and the risk of prion infection in sheep, cattle, and humans. A significant correlation was found in all three prion host species, suggestive of a link between PP development and natural TSE susceptibility [48]. In our experiments, acute *S.* Tm infection did not increase PP size or number, suggesting that the latter are not limiting or do not contribute to the increased risk of prion disease upon *S.* Tm infection.

Previous reports 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 or the kinetics of the scrapie infection [23]. These differences are not surprising, considering that in the present study the inflammation occurs at a mucosal surface where prion uptake would take place. An array of very common inflammatory lesions may therefore alter the dose of prions that may cross 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 upon exposure to prions.

Extensive epidemiologic investigations of vCJD cases have been performed in the United Kingdom to identify risk factors for disease development, 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 increased risk for vCJD [9]. Other than the latter, few exogenous risk factors have correlated with vCJD infection. On the other hand, it may be challenging to recognize any risk posed by small lesions along the gastrointestinal tract (GIT), since (i) lesions may have occurred years prior to prion disease onset, (ii) mild gastrointestinal disease is extremely common, and (iii) 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 GIT erosions and ulcerations caused by infectious and noninfectious diseases, GIT pathology may plausibly exert a marked impact on susceptibility to prion disease in humans and animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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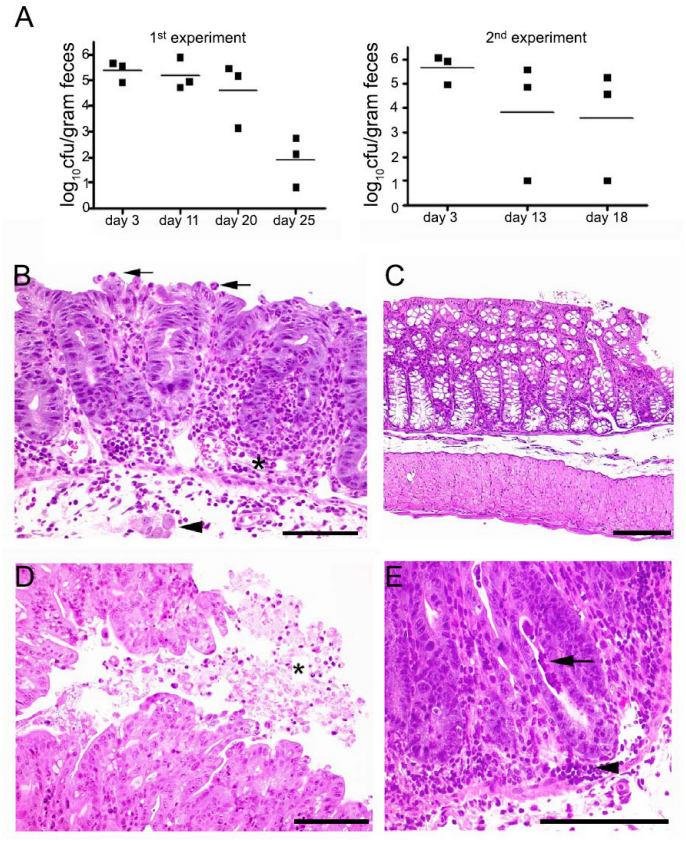

Figure 1.

C57BL/6 mice gavaged intragastrically with *S.* Tm develop colitis. **A**. *S.* Tm colonization was assessed in feces as indicated. In two independent experiments, the mean of *S.* Tm organisms was rapidly reduced to less than 2 log colony forming units (cfu) per gram of feces by 25 days post-infection. **B**. Colon histopathology at 3 days post *S.* Tm challenge shows a widespread influx of neutrophils and some lymphocytes into the lamina propria. Neutrophils were associated with endothelium within vessel lumina (*) and adhered to the mucosal surface (arrows). There was crypt herniation (arrowhead) and a loss of goblet cells. **C**. Normal colon from a PBS control mouse. **D**. Fibrin and suppurative exudate on the colonic mucosal surface (*). **E**. Crypt abscesses (arrow) and pools of inflammatory cells in the mucosa (arrowhead). Scale bar = 100μm.

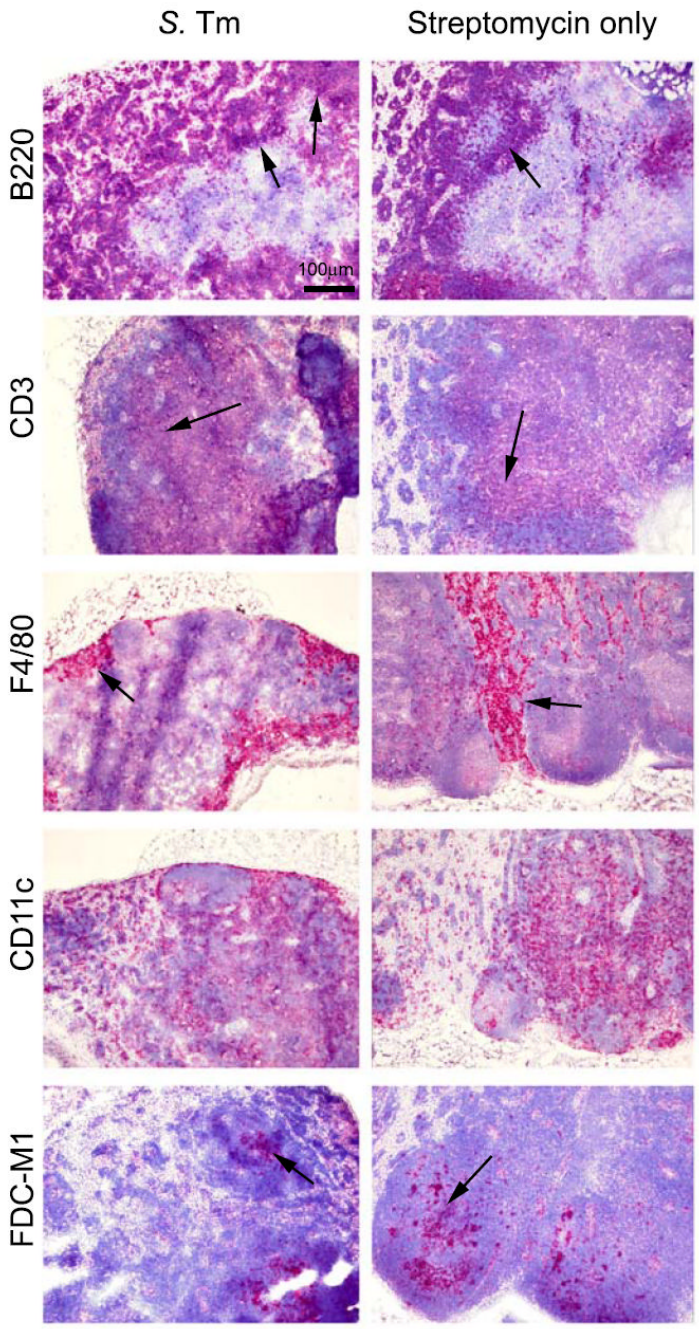

Figure 2.

Immunohistochemical stains on MLN of *S.* Tm-infected mice. Infection was confirmed by fecal bacterial counts. Immunostains for B and plasmacytoid dendritic cells (B220) and T cells (CD3) show that B and T cells are distributed in cortical (arrows) and paracortical areas (arrows), respectively, in both the *S.* Tm and non *S.* Tm-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) were present in the follicular germinal centers in the cortex (arrows), indicating that the overall MLN histology was not dramatically impacted by the *S.* Tm infection.

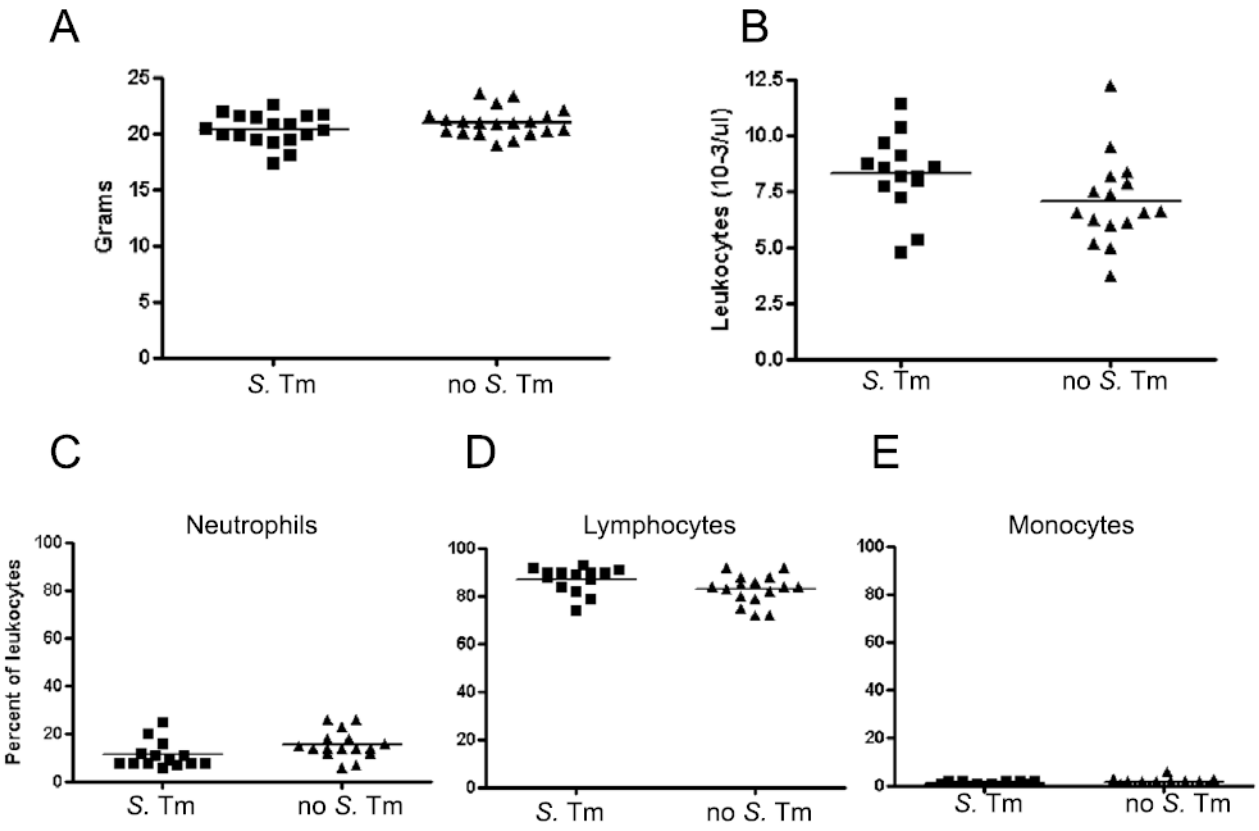

Figure 3.

Mice infected with *S.* Tm (556) reached a normal body weight and had no change in complete blood counts. **A**. Body weight of *S.* Tm-infected and control mice 43 days after prion exposure (Student's unpaired, two-tailed t test, p=0.14). **B**. Total leukocyte counts showed no significant difference between the *S.* Tm and control mice (p=0.08). **C-E**. Differential counts of neutrophils, lymphocytes, and monocytes also showed no or slightly significant differences between *S.* Tm-infected and control mice (p= 0.04, 0.06, and 0.26, respectively).

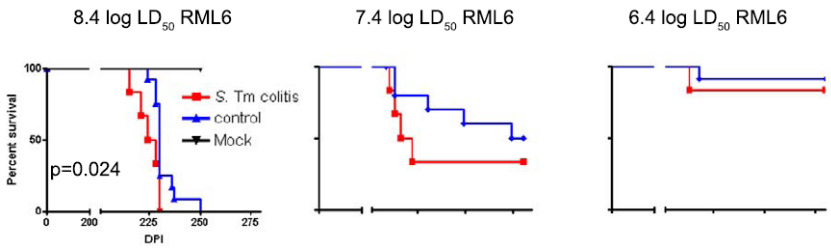

Figure 4.

Survival curves from *S.* Tm and control mice challenged with three doses of prions. Only mice receiving the highest dose of prions showed an attack rate of 100%. The incubation period (days) from prion challenge to the development of terminal scrapie was significantly shorter in *S.* Tm-infected mice than in controls.

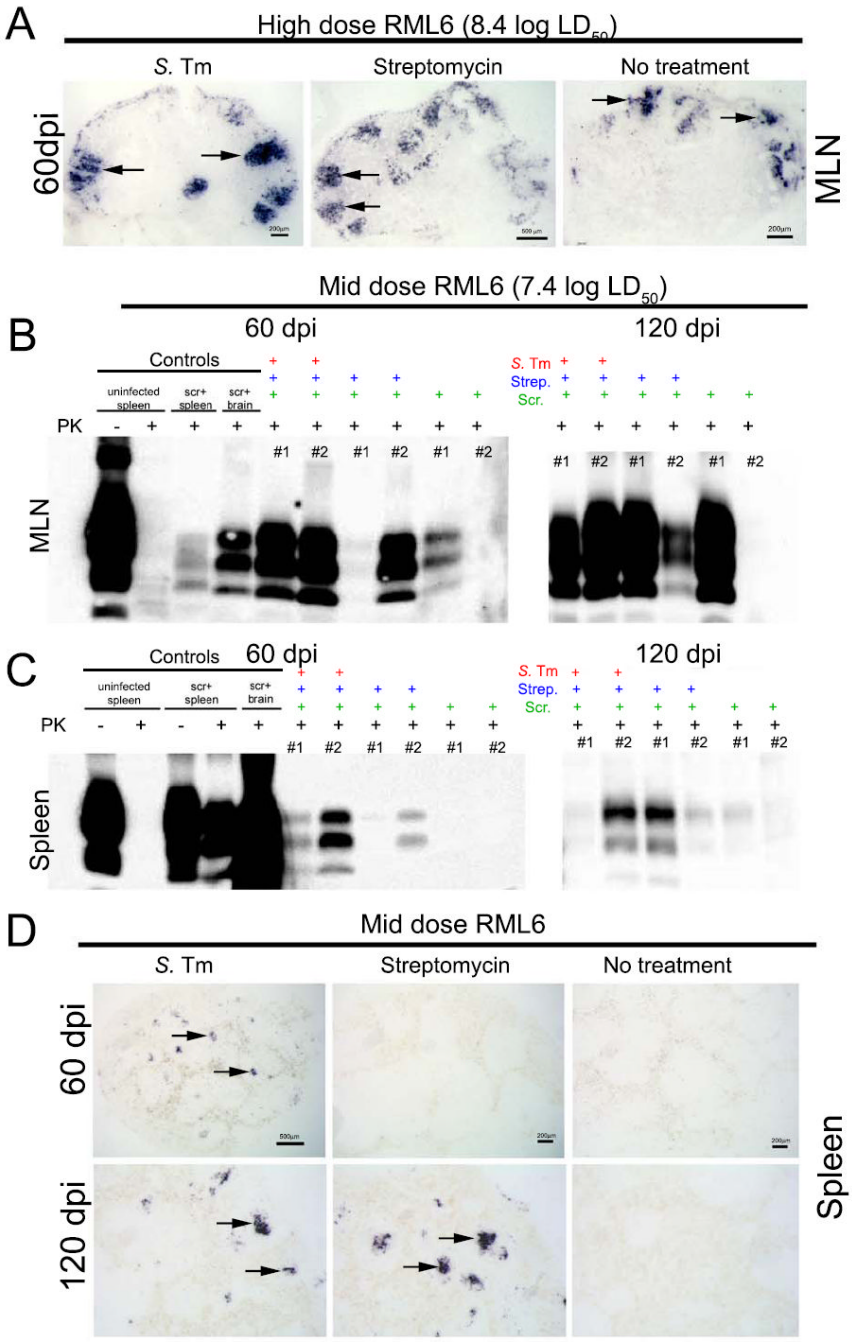

Figure 5.

PrP^{Sc} in MLN and spleen of mice in early prion infection. **A**. Histoblots from *S.* Tm-infected and control mice all show PrP^{Sc} in MLN by 60 dpi (arrows), indicating no difference could be seen in MLN at this timepoint at the highest prion dose. **B**. Western blot of MLN from mice infected with a limiting dose of prions at 60 and 120 dpi indicate that some mice had no detectable PrP^{Sc}, likely due to either slow or absent prion infection. **C**. Spleens from the same mice as in B shows that PrP^{Sc} levels were less than MLN at the same time points. **D**. Histoblots illustrate the dark blue PrP^{Sc} deposition in spleen from the *S.* Tm-infected mouse at 60 dpi (arrows) but not the two non *S.* Tm-infected mice, and in the *S.* Tm and streptomycin-treated

mouse (arrows), but not the second non *S.* Tm-infected mouse at 120 dpi, also indicative of either delayed or lack of prion infection. PK= Proteinase-K treatment of tissue homogenates.

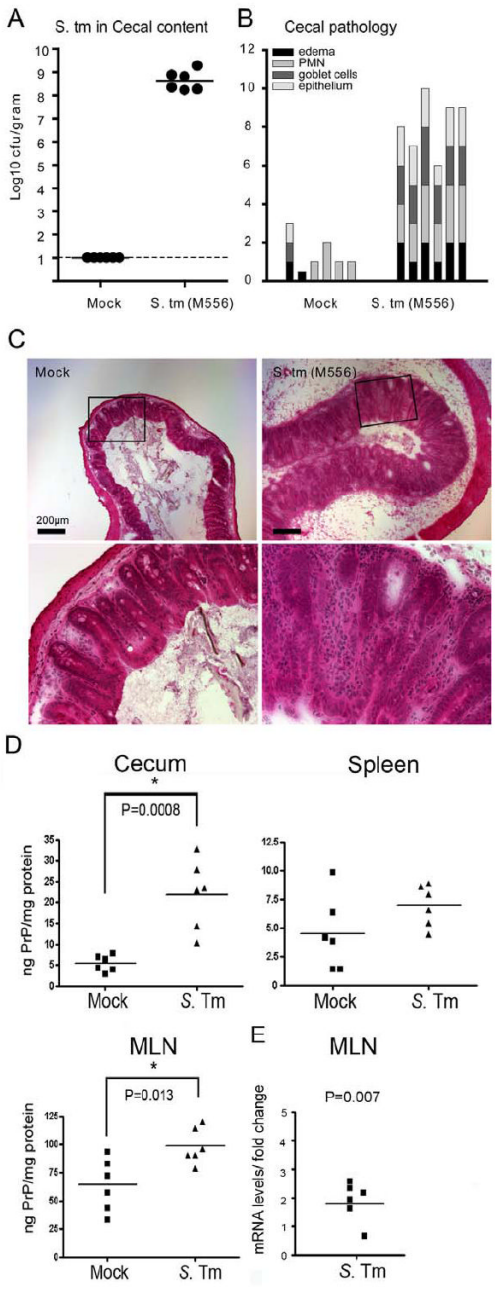

Figure 6.

Streptomycin-pretreated wild type C57BL/6 mice 3 days post-challenge with *S. typhimurium* M556 (*sseD::aphT*; 5×10^7 cfu i.g.; [34]) (n=6) or mock challenged with PBS (n=6). **A**. Mice were sacrificed and cecal *S.* Tm loads were determined by plating on MacConkey agar containing 50µg/ml streptomycin. Dotted line, limit of detection; Black bar, median. **B**. Cecal tissues were embedded and inflammation (or lack thereof) was confirmed by histopathological evaluation of HE-stained tissue sections: tissues were scored with respect to submucosal edema, neutrophil infiltration, loss of goblet cells and epithelial disruption. **C**. Representative images are shown. Left: mock-infected; right: *S.* Tm infected. Scale bar = 200µm. Lower images show enlargement indicated by box in the upper images. **D**. PrP^C ELISA measurements from caecum, MLN, and spleen from mice infected with *S.* Tm and control mice. PrP^C was

elevated in the caecum and MLN, but not spleen due to the SPI-2 mutant *S.* Tm infection. **E.** *Prnp* mRNA levels in MLN were elevated by two-fold in *S.* Tm-infected mice compared with control mice.

Table 1

Incubation periods of RML prion-infected mice.

	oral infection					
	8.4 log LD_{50}		7.4 log LD_{50}		6.4 log LD_{50}	
Colon condition	**Attack rate** ^	**Incubation period**# **(mean ± SE)**	**Attack rate**	**Incubation period**	**Attack rate**	**Incubation period**
Inflammation	6/6 (100%)	225 ± 2	4/6 (67%)	215 ± 3	1/6 (17%)	213, 5x > 560
No inflammation	12/12 (100%)	232±2	5/11* (45%)	236 ± 12	1/11* (9%)	218, 10x > 560

^ Attack rate is shown as the number of mice that develop terminal scrapie over the total number of mice.

Incubation period is defined as the number of days between the prion inoculation and the development of terminal clinical signs of scrapie. Data are mean incubation periods ± SE for diseased animals.

* One mouse died of causes unrelated to scrapie and has not been included in the incubation period calculation.

+ n.d. = not done.