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INFECTION OF C57BL/6 MICE BY *TRYPANOSOMA MUSCULI* MODULATES HOST IMMUNE RESPONSES DURING *BRUCELLA ABORTUS* COCOLONIZATION

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ABSTRACT: Brucellosis, which results in fetal abortions in domestic and wildlife animal populations, is of major concern in the US and throughout much of the world. The disease, caused by *Brucella abortus*, poses an economic threat to agriculture-based communities. A moderately efficacious live attenuated vaccine (*B. abortus* strain RB51) exists. However, even with vaccine use, outbreaks occur. Evidence suggests that elk (*Cervus canadensis*), a wild host reservoir, are the source of recent outbreaks in domestic cattle herds in Wyoming, USA. *Brucella abortus* establishes a chronic, persistent infection in elk. The molecular mechanisms allowing the establishment of this persistent infective state are currently unknown. A potential mechanism could be that concurrent pathogen burdens contribute to persistence. In Wyoming, elk are chronically infected with *Trypanosoma cervi*, which may modulate host responses in a similar manner to that documented for other trypanosomes. To identify any synergistic relationship between the two pathogens, we simulated coinfection in the well-established murine brucellosis model using *Trypanosoma musculi* and *B. abortus* S19. Groups of C57BL/6 mice (*Mus musculus*) were infected with either *B. abortus* strain 19 (S19) or *T. musculi* or both. Sera were collected weekly; spleens from euthanized mice were tested to determine bacterial load near the end of normal brucellosis infection. Although changes in bacterial load were observed during the later stages of brucellosis in those mice coinfected with *T. musculi*, the most significant finding was the suppression of gamma interferon early during the infection along with an increase in interleukin-10 secretion compared with mice infected with either pathogen alone. These results suggest that immune modulatory events occur in the mouse during coinfection and that further experiments are warranted to determine if *T. cervi* impacts *Brucella* infection in elk.

Key words: Brucellosis, coinfection, immune modulation, trypanosomiasis.

INTRODUCTION

Brucellosis is caused by *Brucella abortus*, a gram-negative intracellular bacterium that can infect a variety of mammalian hosts, usually resulting in abortion or production of weak offspring. In Wyoming USA, brucellosis is endemic in wild elk (*Cervus canadensis*), which present a potential risk to free-ranging cattle populations, creating the potential for economic and production losses (Higgins et al. 2012; Roberts et al. 2012; Cross et al. 2013). Elk develop a chronic disease state and serve as a reservoir that maintains *B. abortus* in the environment (Cook et al. 2002). At one major winter feed ground near Jackson, Wyoming there was a *B. abortus* antibody prevalence of 21.9% in more than 3,300 animals sampled over several years (Scurlock and Edwards 2010). At adjacent winter feed grounds there was an antibody prevalence of approximately 3.7% in approximately 3,800 animals tested (Scurlock and Edwards 2010). These elk herd prevalence data support concern regarding the ability of elk herds to transmit the disease to domestic, free-ranging cattle.

Elevated IL-10 may contribute to the establishment of the chronic infective state and may play an important role in pathogenesis (Baldwin and Goenka 2006). The identification of the *B. abortus* protein PrpA as a nonspecific B-cell mitogen and inducer of IL-10 has

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strengthened this hypothesis (Montes et al. 2002; Spera et al. 2006). Through this proliferation and IL-10 secretory events, it confers a specific advantage on the pathogen, allowing it to avoid specific and directed humoral and cell-mediated immune responses, leading to modulation of the host response (Palanivel et al. 1996; Spera et al. 2006). Some other endemic pathogens in elk are thought to utilize a similar mechanism.

Cervids in Wyoming are chronically infected with *T. cervi*. Surveys in the 1970s showed that 71% of free-ranging elk in western Wyoming were infected with *T. cervi* (Kingston et al. 1981). *Trypanosoma cruzi* and other blood parasites have been implicated in inducing chronic infections by modulating the host immune response via mechanisms such as those mediated by PrpA (Palanivel et al. 1996; Montes et al. 2002; Chamond et al. 2005). We propose that *T. cervi* acts similar to other trypanosomes in suppressing immune activation. This hypothesis is supported by the identification of the immunomodulatory role of PrpA from *T. cruzi*. Modulation of immunity by this mechanism is not unique to *T. cruzi* and *B. abortus*. In other related blood parasitic pathogens (*Leishmania* spp. and *Schistosoma* spp.) there is also a nonspecific polyclonal B-cell proliferation event that contributes to the maintenance of the pathogen in vivo by down-regulating the humoral and cell-mediated immune response (Palanivel et al. 1996). Observations reveal no apparent pathologic changes in elk due to *T. cervi* infection, suggesting more-subtle effects on the host by the parasite (Kingston et al. 1981). It is possible, therefore, that immune modulatory effects of trypanosomes on elk by the mechanism described above allows the establishment of a *B. abortus* chronic infection.

The most important evidence to support immune cross-regulation, resulting in a negative effect on vaccination, comes from a study in which mice were vaccinated against *Trichinella spiralis* and subsequently infected with *T. spiralis* and *Trypanosoma brucei*. Animals infected with both pathogens had decreased IL-5 secretion, higher worm burdens, decreased eosinophil response, and decreased formation of IgG1 to *T. spiralis* (Onah and Wakelin 2000). Even in animals vaccinated against *T. spiralis*, the immune response was blunted and markers of inflammation such as IL-5 were decreased during coinfection (Onah and Wakelin 2000). The authors concluded that coinfection with *T. brucei* negatively affects the ability of the host to respond, even with previously high circulating IgG antibody titers to *T. spiralis* (Onah and Wakelin 2000).

We built on the concept of coinfective synergism by modeling *B. abortus* and *T. cervi* interplay in naïve C57BL/6 mice. The murine model for *B. abortus* is well established and allows for efficient evaluation of the effect trypanosomes have on the host immune system (Montaraz and Winter 1986; Grillo et al. 2012). *Trypanosoma musculi* was also evaluated in C57BL/6 mice and found to induce profound T-cell depression and anergy at 7 days postinfection (dpi) (Magluilo et al. 1983). This observation supports the hypothesis that even "nonpathogenic" trypanosome species can induce some level of immune modulation. The potential for elk to be actively infected by both organisms (*B. abortus* and *T. cervi*), which are suspected immune modulators, could explain differences in the biology of disease in elk compared to domestic cattle.

We infected 60 C57BL/6 mice with *T. musculi* and, following parasitism, infected them with *B. abortus* S19 at 7 dpi and 28 dpi with *T. musculi*. After infection with both organisms, immune response was evaluated by measuring serum concentration of IFN-γ, IL-10, IgG, and IgM. Additionally, organism burden was measured by number of colony-forming units (CFU) per spleen at the end of the experiment. The design of experiments in this study, using a murine model,

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addresses the potential for trypanosomes to have an impact on *B. abortus* biology, more specifically on the host immune response, offering insight into the possibility of an effect that may be occurring in Wyoming elk.

METHODS

Bacterial and parasite strains

Brucella abortus strain 19 was generously donated by the Colorado Serum Company (Denver, Colorado, USA) and grown and maintained on blood agar. Working cultures, grown in brain-heart infusion broth, were quantitated by serial dilution and plating after determining optical density correlates at 600 nm. *Trypanosoma musculi* CDC strain was obtained from ATCC (Manassas, Virginia, USA) and maintained in Roswell Park Memorial Institute (RPMI) medium with PenG/streptomycin (Invitrogen, Carlsbad, California, USA) antibiotics with 10% fetal bovine serum (Invitrogen); dose was determined counting dividing forms (DF) using a hemocytometer.

Animal studies

Animals used in this study were cared for according to strict adherence to the policies and regulations established by the US Public Health Service "Humane Care and Use of Laboratory Animals"(US Public Health Service 2002) and an approved animal protocol from the University of Wyoming Institutional Animal Care and Use Committee (DHHS Assurance A3216-1).

Sixty C57BL/6 mice were infected via an intraperitoneal route (i.p.) with 1×10^6 DF *T. musculi* organisms; parasitemia was verified on the third day postinfection by i.p. lavage of three mice per group (nine total) with RPMI medium and evaluated on microscopic wet mount. The 60 mice were divided into three groups of 20 mice. One group was designated as "*T. musculi*-only" and no additional infections were performed. A second cohort of 20 mice was infected at 7 dpi with *T. musculi* with 5×10^4 *B. abortus* S19 CFU i.p. (Group: "7-day coinfected"). The last 20 mice were infected at 28 dpi with 5×10^4 *B. abortus* S19 CFU i.p. (Group: "28-day coinfected"). An additional 20 mice not infected with *T. musculi* were infected with 5×10^4 *B. abortus* S19 CFU i.p. only to serve as a control (Group: "S19 Only"). Five mice from each group were euthanized at 42, 56, 63, and 70 dpi. The spleens were removed and tissue homogenates (diluted) were incubated on blood agar plates to determine bacterial CFU per spleen. An additional 10 mice served as negative controls for sera. Every week, three mice per group were bled by retro-orbital puncture to obtain serum for cytokine and immunoglobulin assays. All data are represented as dpi with *B. abortus* S19, with the exception of the *T. musculi*-only group which is dpi with *T. musculi*.

Immunologic studies

Three mice, chosen at random from each group, were bled and approximately 100 μL of serum were recovered and diluted 1:3 in 1X phosphate-buffered saline for use in cytokine and immunoglobulin assays. IL-10 and IFN-γ serum concentrations were assessed in all mouse groups and compared with nominal baseline levels in naïve animals. Sera were analyzed by QuantiKine® enzyme-linked immunosorbent assay (ELISA; R&D Systems, La Jolla, California, USA) for levels of IL-10 and IFN-γ to determine immunologic response. Additionally, concentrations of IgM and IgG antibodies were assayed with the Mouse Quantitative IgG (and IgM) ELISA kit (Bethyl Laboratories, Montgomery, Texas, USA) from the same sera. All ELISAs were performed in triplicate.

Statistical analysis

Statistical analyses were performed in SAS (SAS Corporation, Cary, North Carolina USA) comparing mean IL-10, IgG, IgM, and IFN-γ concentrations as well as

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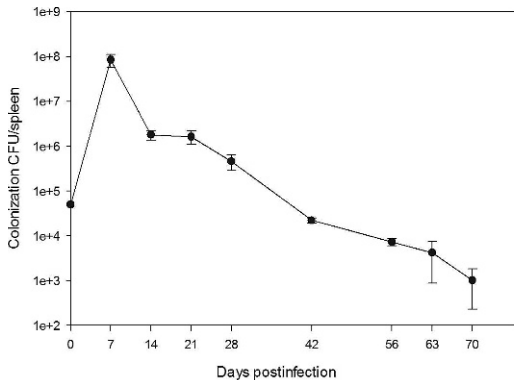

FIGURE 1. Colonization of *Brucella abortus* S19 in splenic tissue of C57BL/6 mice. Mice (n=24) infected with 5×10^4 colony-forming units (CFU) of *B. abortus* S19 and sacrificed at eight time points following infection. Each data point represents the average (n=3) CFU per spleen from mice. Error bars indicate standard deviation.

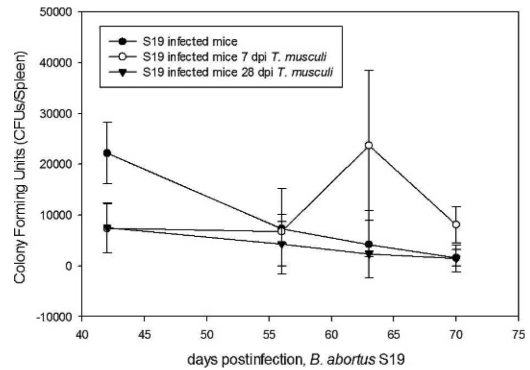

FIGURE 2. Increased bacterial spleen colonization during late infection following coinfection of C57BL/6 mice with *Trypanosoma musculi* and *Brucella abortus*. Mice infected with 1×10^6 dividing forms of *T. musculi* or 5×10^4 colony-forming units (CFU) of *B. abortus* S19, or both. Each data point represents the average CFUs/spleen from mice (n=5) in each group. Error bars indicate standard deviation.

mean CFU/spleen at each time point using an analysis of variance followed by Fisher's least significant difference for post hoc comparisons.

RESULTS

S19 infection kinetics in C57BL/6 mice

Although established in BALB/c mice, previous studies have not evaluated the infection kinetics of *B. abortus* S19 in C57BL/6 over a 70-day period. We infected 24 C57BL/6 mice with 5×10^4 CFU *B. abortus* S19 i.p. Three mice were sacrificed at 7, 14, 21, 28, 42, 56, 63, and 70 dpi and CFU were counted at each time-point (Fig. 1). In contrast to BALB/c mice (Montaraz and Winter 1986), C57BL/6 animals remained colonized with S19 at a moderate level 70 dpi.

Bacterial load in coinfected *B. abortus* S19 and *T. musculi* C57BL/6 mice

At 42 dpi mice coinfected with *B. abortus* S19 and *T. musculi* had lower bacterial loads than mice infected with *B. abortus* S19 only (P=0.0005; Fig. 2). At both 63 and 70 dpi with S19, however, mice from the group infected with both *T. musculi* and *B. abortus* S19 (7-day coinfected) had significantly (P=0.0378 for 63 dpi and P=0.0030 for 70 dpi) higher levels of colonization than mice infected with S19-only or those infected first with *T. musculi* followed by S19 28 days later (28-day coinfected group; Fig. 2).

Cytokine response to infection

Interleukin-10, a primary anti-inflammatory cytokine, was detected in all experimental groups while naive controls animals had no detectable IL-10 throughout the experiment, with one exception at 49 days. The naïve animals at the 49-day time point had an average of 21.8 μg/mL (SD 0.29 μg/mL) of IL-10 in sera. The highest levels of IL-10 were measured at 7 dpi and 14 dpi with S19 in both of the coinfected groups (P<0.00001 for both time points) compared to control groups (naïve, S19 and *T. musculi*-only infected mice; Fig. 3). In the 28-day coinfected group a significantly greater (P<0.00001) amount of IL-10 (212.6 μg/mL, SD 10.70) was also detected 21 days after S19 infection compared to control groups (naïve, S19 and *T. musculi*-only infected mice) and the other coinfected group (7-day), which all had IL-10 levels below the

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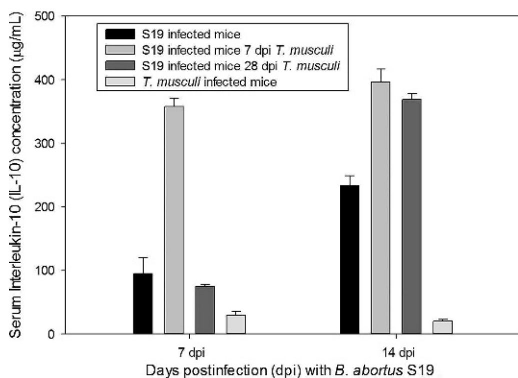

FIGURE 3. Elevated IL-10 response following coinfection of C57BL/6 mice with *Trypanosoma musculi* and *Brucella abortus*. Mice infected with 1×10^6 dividing forms of *T. musculi* or 5×10^4 colony-forming units of *B. abortus* S19, or both. Sera were collected from three randomly selected mice from the group of 20 weekly. Each data point represents the average IL-10 concentration from sera from selected mice (n=3). Error bars indicate standard deviation.

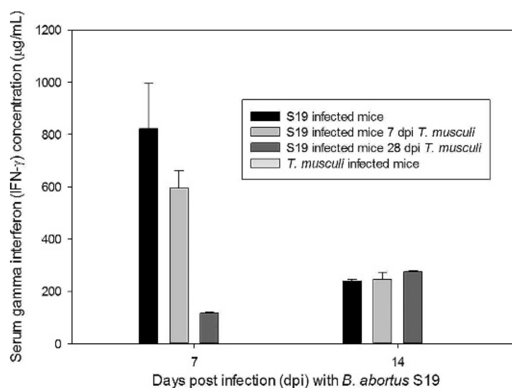

FIGURE 4. Suppression of IFN-gamma response in mice during coinfection with *Trypanosoma musculi* and *Brucella abortus*. C57BL/6 mice infected with 1×10^6 dividing forms of *T. musculi* or 5×10^4 colony-forming units of *B. abortus* S19, or both. Sera were collected from 3 randomly selected mice from the group of 20 weekly. Each data point represents the average IFN-gamma concentration from sera from selected mice (n=3). Error bars indicate standard deviation.

test detection threshold (data not shown). Infection with *T. musculi* alone did not induce a marked IL-10 response (Fig. 3). No significant differences in levels of IL-10 (>40 µg/mL; P>0.05) were detected through the remainder of the experiment in any mouse (28–70 days).

Gamma interferon was detected in all the groups except for the mice infected with *T. musculi* alone and the naive control animals. Significant differences in IFN-γ serum concentrations were observed at 7 dpi and 21 dpi with S19 (Fig 4; 21-day data not shown). At 7 dpi all three groups (S19, 7-day, and 28-day coinfected) differed significantly (P< 0.00001) from each other with the 28-day coinfected mice having the lowest average concentration of IFN-γ (Fig. 4). At 14 dpi with S19, all three groups (S19, 7-day, and 28-day coinfected) were not statistically different (P=0.0806) and displayed a similar amount of IFN-γ in blood (Fig. 4). At 21 dpi with S19, the 28-day S19 coinfected animals had significant concentrations (P<0.00001) of IFN-γ (93.76 µg/mL, SD 16.76 µg/mL) compared to mice infected with S19 alone and the 7-day coinfected animals (data not shown). Mice infected with *T. musculi* by itself contained no IFN-γ in serum samples. No significant levels of IFN-γ (>40 µg/mL and P<0.05) were detected through the remainder of the experiment in any mouse (28–70 days).

Serum immunoglobulin concentrations

Concentration of serum IgM was assessed for each animal group at 7, 14, and 42 dpi with S19 by quantitative ELISA. Mice coinfected with *T. musculi* and 7 days later with *B. abortus* S19 had a statistically significant (P<0.00001 for 7, 14, and 42 dpi) lower IgM concentration than did controls (naïve, S19, and *T. musculi*-only infected animals) and the other coinfected group (28-day group) at 7, 14, and 42 dpi with S19 (Fig. 5). At 7 dpi, S19-only infected mice had a marked increase in serum IgM compared to all the other groups (highest P=0.0020, 28-day coinfected mice). At 14 dpi *T. musculi* infected-only mice had statistically reduced IgM concentrations compared to the other experimental groups (highest P=0.0225, 7-day coinfected mice) except

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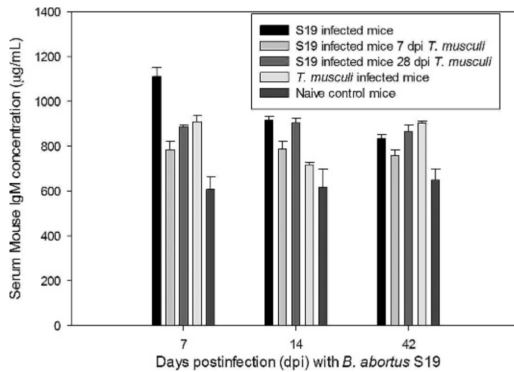

FIGURE 5. Modulation of host IgM response following coinfection of mice with *Trypanosoma musculi* and *Brucella abortus.* C57BL/6 mice infected with 1×10^6 dividing forms of *T. musculi* or 5×10^4 colony-forming units of *B. abortus* S19, or both. Sera were collected from three randomly selected mice from the group of 20 weekly. Each data point represents the average IgM concentration from sera from selected mice ($n=3$). Error bars indicate standard deviation.

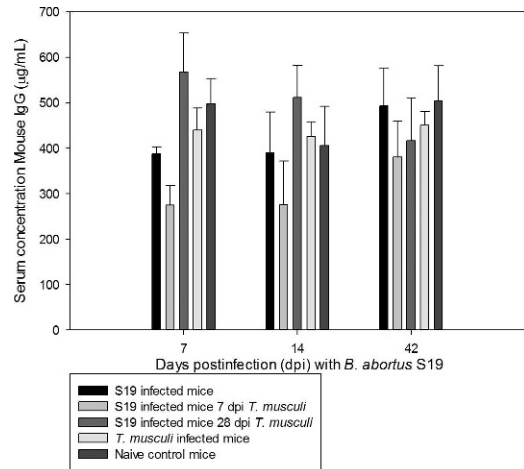

FIGURE 6. Reduction of serum IgG following coinfection of mice *Trypanosoma musculi* and *Brucella abortus.* C57BL/6 mice infected with 1×10^6 dividing forms of *T. musculi* or 5×10^4 colony-forming units of *B. abortus* S19, or both. Sera were collected from three randomly selected mice from the group of 20 weekly. Each data point represents the average IgG concentration from sera from selected mice ($n=3$). Error bars indicate standard deviation.

for naïve animals ($P=0.0992$) where no difference was noted. IgM concentrations at 42 dpi showed a difference in coinfected mice at 7 days versus all other groups (highest $P=0.0278$, naïve mice). All experimental groups had statistically significant higher levels of IgM serum concentrations compared to naïve animals throughout all time points assessed (highest $P=0.042$, *T. musculi* infected mice), with one exception at 14 days with *T. musculi*-only infected mice.

Immunoglobulin G concentrations were also determined for mice at 7, 14, and 42 dpi with *B. abortus* S19. Mice in the acutely coinfected (7-day mice) group had significantly decreased serum concentrations of IgG at 7 dpi with S19 compared to naïve animals and other infected animals (highest $P=0.0127$; S19-only mice, Fig. 6). Mice at 14 dpi or 42 dpi did not differ significantly ($P=0.0497$ and $P=0.221$, respectively).

DISCUSSION

Our observations on apparent immune-suppressive effects in C57BL/6 mice during trypanosomal infection are consistent with earlier studies (Magluilo et al. 1983). Furthermore, our results on coinfection with two pathogens correlate well with the previously published data that showed *T. brucei* promotes higher levels of a second pathogen (*Trichinella spiralis*) in the host when coinfected (Onah and Wakelin 2000). Although we did not detect a significant difference in experimental mice until 63 dpi and 70 dpi, additional differences in splenic colonization may be evident early in infection. In addition, suppression of IgG antibody production was consistent between our study and that of the *T. spiralis*-*T. brucei* study in that both models induced IgG suppression. We detected pronounced suppression at 7 dpi; the *T. spiralis*-*T. brucei* study did not measure IgM production (Onah and Wakelin 2000). One major difference between the two studies was the INF-γ response. In the *T. spiralis*-*T. brucei* coinfection group, significantly elevated levels of INF-γ were observed

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compared to control groups and mice infected with either pathogen alone (Onah and Wakelin 2000). This may be due to the different *Trypanosoma* species used and because *T. brucei* induces a greater inflammatory response than does *T. musculi*.

Despite genetic differences of *B. abortus* S19 from wild-type strains commonly isolated in nature (Crasta et al. 2008), S19 has been shown to cause disease in mice that is similar to that in large animals, especially in the reproductive tract, supporting its utility as an effective model (Enright et al. 1990; Kim et al. 2005). The selection of *T. musculi*, the patho-adapted species of trypanosome for mice, was based on the benign nature which appeared similar to reports of *T. cervi* in elk (Albright and Albright 1981). The use of *T. cervi* in mice was initially proposed for the current study. *Trypanosoma cervi* derived from elk blood, however, could not be maintained in culture for more than a few days in the laboratory, regardless of medium used. Additionally, the use of a mouse model is less preferable than a cervid model; however, because of the expense and regulations on using cervids in research, demonstrating proof-of-concept in a mouse model is necessary prior to attempting to transition to a large-animal model.

Our data strongly support the hypothesis that coinfection with microorganisms known to suppress the immune response alters the ability of the host to effectively clear a bacterial pathogen. Specifically, trypanosome-associated immune suppression negatively affects the host immune response to *B. abortus*. This finding adds credence to the hypothesis that *T. cervi* (or other indigenous microorganisms), by altering the appropriate memory response to *B. abortus*, is contributing to the failure of the RB51 vaccination campaign. Vaccination of elk with either of the two approved live attenuated vaccines (*B. abortus* strains RB51 and S19) for cattle has been unsuccessful in cervids (Cook et al. 2002; Olsen et al. 2002, 2006; Olsen 2010). Failure of both RB51 and S19 to induce protective memory responses in elk is not well understood and is further confounded by the observation that elk appear to respond initially to vaccination. Despite this observation, analysis of peripheral blood mononuclear cells recovered from vaccinated animals and exposed to γ-irradiated S19 or RB51 showed no change in IFN-γ production compared to controls (Olsen et al. 2006). This finding suggests that antigens associated with S19 or RB51 are unable to stimulate proinflammatory elements of the adaptive immune system and appear to be related to failure of immune memory formation (Olsen et al. 2006). The elk vaccination studies also showed that elk do, in fact, develop a robust humoral response to vaccination, though some consider the humoral response insufficient to mediate clearance (Olsen et al. 2006). The failure of the elk vaccination program in Wyoming has likely allowed *Brucella* to continue to persist in the Greater Yellowstone area.

In the mouse model, elevated levels of IL-10 have been shown to prolong *B. abortus* infection (Fernandes and Baldwin 1995). In our study mice infected with *T. musculi* alone did not display significant levels of IL-10. Hence, there may be other anti-inflammatory pathways by which *T. musculi* promotes immune suppression. In previous studies the origin of *T. musculi* immune suppression and a profile of the cytokine response were not identified (Albright et al. 1978; Magluilo et al. 1983). The INF-γ data collected also correlates well with our model and the known effects of *T. musculi*. At 7 dpi S19, a significant reduction in the amount of IFN-γ produced was observed in the coinfected groups compared to controls. *Trypanosoma musculi* also induces marked T-cell suppression in C57BL/6 mice at 7 dpi and 14 dpi, which we observed indirectly with IgG in the mice in our study (Magluilo et al. 1983; House and Dean 1988; Albright and Albright 1989). At 14 dpi we observed stabilization

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of IFN-γ production across the groups, suggesting the immune system began to overcome the suppressive effects of *T. musculi*, which is consistent with the start of clearance beginning at 21 days. Early in infection, mice infected with *T. musculi* and 28 days later (after clearance) with *B. abortus* displayed little IFN-γ production at 7 dpi. This curious finding could be attributed to chronic kidney colonization by *T. musculi* and may represent strong immune cross-regulation, in that immunosuppression from *T. musculi* is negating the initial inflammatory response induced by S19 infection (Stevens et al. 1995; Monroy and Dusanic 2000). Mice infected with *T. musculi* and 28 days later with *B. abortus* displayed an anomalous and significant amount of IFN-γ at 21 dpi with S19. It is quite possible that, in these mice, reactive antigens from *B. abortus* were upregulated and triggered a response or that immunomodulatory events induced by *T. musculi* were overcome by the host. Both of these findings in the group of animals thought to have cleared *T. musculi* warrant further investigation to identify the source of the suppressive and stimulatory events.

We also measured immunoglobulin levels in mice. The IgM responses at 7 dpi were very high in S19-infected mice, moderate in the trypanosome and 28-day coinfection group, and significantly lower in the 7-day coinfection group (Fig. 5). As predicted, an early humoral response was suppressed by coinfection with trypanosomes. At 14 days, the S19-infected 7 dpi *T. musculi* group again had low serum IgM concentrations compared to all other groups (naïve, S19 and *T. musculi* alone, and 28 dpi coinfected). This decrease in serum IgM concentration is likely due to T-cell anergy induced by the trypanosome on the host at this time point (Magluilo et al. 1983). Data from 42 dpi again showed a decrease in IgM production from the coinfected animals at 7 days, which was consistent throughout the course of coinfection. This supports the hypothesis that coinfection with these two species modulates the host's ability to mount a normal immune response.

Immunoglobulin G data correlated well with previous *T. brucei* and *Trichinella* spp. studies (Onah and Wakelin 2000). Decreased concentrations of serum IgG were maximally appreciated at 7 days in mice coinfected with S19 and *T. musculi* (7 dpi coinfected group), complementing the cytokine data and previous reports (Magluilo et al. 1983; Onah and Wakelin 2000). The S19 also displayed early suppressive effects at 7 dpi before returning to concentrations consistent with naïve animals. The S19 IgG suppression is consistent with previous reports in this mouse strain (Grillo et al. 2012).

Microorganisms recovered from spleen homogenates revealed an interesting pattern in that differences among the coinfected groups versus S19 alone were not appreciated until 70 dpi. Unfortunately, due to the use of the mice for serial retroorbital sera collection, we were unable to obtain earlier colonization data where perhaps more significant changes in splenic colonization by S19 may have been observed. It is possible that early heightened bacteremia is concomitant with the presence of increased IL-10 early during infection. Another explanation for the observation of increased S19 colonization later during infection is that *T. musculi* can chronically colonize renal arteries after clearance of systemic parasitemia (Monroy and Dusanic 2000). This chronic colonization of kidney forms of *T. musculi* could be exerting immunosuppressive effects which in turn could slow splenic clearance of *B. abortus*. To investigate the possibility of chronic kidney colonization by *T. musculi*, we cultured the kidneys from the coinfected 7 day group animals from 63 dpi and 70 dpi S19, but this did not yield any viable *T. musculi* nor was any visualized on impression smears made from the kidney. Thus, the mechanism for the increase in colonization later during infection remains unclear.

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Results of our study suggest that coinfection of a host with two organisms that are known to exhibit immune suppression activity could alter the infection kinetics of at least one and lead to longer duration of colonization of one or both organisms in the host. This phenomenon is likely not unique to the two pathogens studied. A recent example includes the African buffalo (*Syncerus caffer*), which has seen an emergence of bovine tuberculosis linked to nematode immunosuppression. The mechanism described is a shift toward a Th2-dominated response, driven by helminth immune suppression, leading to favorable colonization conditions for tuberculosis (Ezenwa et al. 2010). Studies such as these complement the earlier experimental study describing a *Trypanosoma*-induced immune suppression effect on *Trichinella* worm burdens and decreased vaccine efficacy (Onah and Wakelin 2000). Evaluation of the effect of trypanosomes (or other pathogens) on vaccination against brucellosis, followed by challenge, should further clarify the influence of other pathogen burdens on the host. The implications of this study are that the possibility exists for supposed benign parasitic colonization to enhance infection of a disease-producing organism in a synergistic manner. Application of these findings extends beyond elk in Wyoming infected with *T. cervi* and *B. abortus*. Officials in developing countries with vaccination-based eradication campaigns should consider how other microorganisms may alter host immunity as well as the ability of the host to manage disease burdens effectively.

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