Interleukin-12p70–Dependent Interferon-γ Production Is Crucial for Resistance in African Trypanosomiasis

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African trypanosomiasis encompasses diseases caused by pathogenic trypanosomes, infecting both humans and animals. In the present article, we dissected the possible role of members of the interleukin (IL)–12 family during infection with *Trypanosoma brucei brucei* and *Trypanosoma evansi* in mice. IL-12p35$^{-/-}$, IL-12p40$^{-/-}$, and IL-12p35$^{-/-}$/p40$^{-/-}$ mice were susceptible to both pathogens, as was demonstrated by the increased mortality among these mice, compared with wild-type C57BL/6 mice. The different IL-12p70$^{-/-}$ mouse strains showed similar mortality kinetics, suggesting that IL-12p70—but not the IL-12p80 homodimer or IL-23— plays a crucial role in survival. Although there were similar plasma levels of immunoglobulin (Ig) M and IgG2a in IL-12–deficient mice and wild-type mice, interferon (IFN)–γ production, especially during early infection, was severely impaired in all IL-12p70$^{-/-}$ mouse strains, demonstrating an IL-12p70–dependent mechanism for IFN-γ production. Because IFN-γ receptor–deficient mice (IFN-γR/$^-$) were also highly susceptible to both *Trypanosoma* species, IL-12p70–dependent IFN-γ production seems to be the important mechanism involved in resistance against both pathogens.

Trypanosomes are unicellular eukaryotic parasites that replicate freely in the bloodstream of a mammalian host, ultimately resulting in sleeping sickness in humans, "nagana" (due to *Trypanosoma brucei brucei*) in cattle, or "surra" (due to *Trypanosoma evansi*) in various other animals [1].

Trypanosomes regulate and escape the B and T cell responses of the host. The foremost mechanism is antigenic variation, whereby the surface coat of the trypanosome consisting of variant surface glycoprotein (VSG) is regularly replaced, thus continually evading the immune response of the host [2, 3]. At the time of infection, the host responds with rapid B cell activation, which has been found to be important for clearance of parasites from within vascular regions [4, 5]. Furthermore, a rapid Th1 response is initiated [6–9], with the characteristic production of interleukin (IL)–12 and interferon (IFN)–γ [10]. Results from Mansfield and Paulnock [11] suggested that initiation of a polarized VSG-specific Th1 cellular response is dependent on the presence of IL-12 and IL-18.

The IL-12p70 molecule is a heterodimeric protein composed of 2 glycosylated subunits—namely, p35 and p40 [12, 13]. The p40 subunits also form homodimers (i.e., IL-12p80) and may act antagonistically to the functioning of IL-12 by blocking signal transduction via the receptor [14]. However, IL-12p80 homodimer may also act in an agonistic manner to IL-12 in its ability to induce cell-mediated Th1 immune responses [15, 16]. In addition to forming p35/p40 and p80 dimers, the p40 subunit also binds to p19, forming

IL-23 [17]. The IL-12p80 homodimer also acts as an antagonist in IL-23 signaling [14].

Studies using IL-12–deficient mice in models of severe infectious disease, such as toxoplasmosis [18], listeriosis [19], leishmaniasis [20], Chagas disease [21], and tuberculosis [16], have found that IL-12–independent IFN-γ can be produced, resulting in residual resistance. IFN-γ, which plays a central role in resistance to trypanosomiasis, is produced primarily by activated Th1 cells, which, in turn, induce macrophages to release trypanocidal molecules, including reactive oxygen intermediates, reactive nitrogen intermediates, and tumor necrosis factor (TNF) [7, 8, 22, 23]. Mice lacking TNF have displayed reduced ability to control the parasite load in the vascular compartment [24].

In this comparative study of *T. b. brucei* and *T. evansi* infection models, the IL-12 family—namely, IL-12p70, IL-12p80 homodimer, and IL-23—were examined using IL-12p35–, IL-12p40–, and IL-12p35/p40–deficient mice. The data obtained suggest that IL-12p70—but not IL-12p80 homodimer or IL-23—is necessary for the development of resistance against both *T. brucei* and *T. evansi* parasites. IL-12 deletion resulted in the impaired IFN-γ production necessary for parasitemia control and survival.

MATERIALS AND METHODS

Mice. IL-12p35$^{-/-}$ [20] and IL-12p40$^{-/-}$ [25] on a 129Sv/Ev background, backcrossed 5 times to C57BL/6, IFN-γ receptor (IFN-γR)$^{-/-}$ 129Sv/Ev [26], C57BL/6, and 129Sv/Ev mice were maintained under specific pathogen–free conditions. For the generation of homozygous double-deficient mice, IL-12p35$^{-/-}$ and IL-12p40$^{-/-}$ mice were intercrossed and genotyped by polymerase chain reaction [20, 25]. Experiments were performed within the guidelines of the Animal Research Ethics Board of the University of Cape Town (Cape Town, South Africa) (animal ethics approval number 005/041).

Parasites. The pleomorphic *T. b. brucei* AnTat 1.1E clone and *T. evansi* (code, KETRI 2480; stock no., ITMAS 110297) were obtained from the Institute of Tropical Medicine (Antwerp, Belgium).

Infection and parasitemia. Mice were infected intraperitoneally with 5×10^3 *T. b. brucei* trypanosomes and 1×10^3 *T. evansi* trypanosomes. Blood samples were obtained for parasite enumeration, diluted 1/200 in RPMI 1640 (10% fetal calf serum) (Highveld Biological, Johannesburg), and counted under a light microscope (Nikon).

Cytokine ELISAs. Cytokines in plasma were measured by ELISA performed as described elsewhere [27], with modifications. Plasma and cytokine standards were serially diluted 3- and 2-fold, respectively. Cytokine standards, together with coating and biotinylated antibodies for TNF and IFN-γ (BD PharMingen), were used. Streptavidin-labeled horseradish per-

oxidase (BD PharMingen) and 3,3′,5,5-tetramethylbenzidine (KPL) were used for detection and development, respectively. The lower limit of detection for both IFN-γ and TNF was 16 pg/mL.

Quantification of anti-VSG plasma titers. Anti–trypanosome-derived antigen antibody titers were determined as described elsewhere [28].

Graph and statistical analysis. Data are expressed as the mean ($\pm$SE) of individual determinations, with statistical analysis performed using the unpaired Student's *t* test for ELISAs. Survival data showed that, for each experiment, mortality in >1 IL-12–deficient group was significantly increased, compared with mortality in wild-type mice, by use of Kaplan-Meier analysis with the log-rank test. Thus, experiments were pooled, increasing the number of mice per group. Analysis between wild-type groups of repeated experiments performed using 1-way analysis of variance (ANOVA), with Dunnett's test performed as the posttest, found that parasitemia was not significantly different between experiments, and, thus, data were pooled. At each time point, the pooled parasitemia was analyzed using 1-way ANOVA, with Dunnett's test performed as a posttest and Student's *t* test performed to compare IL-12– and IFN-γR–deficient mice with their wild-type controls, respectively.

RESULTS

Reduced survival among IL-12p35$^{-/-}$/p40$^{-/-}$, IL-12p40$^{-/-}$, and IL-12p35$^{-/-}$ mice infected with T. b. brucei ***and*** T. evansi. To determine the role of IL-12p70 (p35/p40), IL-23 (p19/p40), and IL-12p80 homodimer during trypanosomiasis, mice deficient in either the p35 gene, the p40 gene, or both were infected with *T. b. brucei* and *T. evansi* and were monitored for survival (table 1). The duration of survival was significantly decreased in all *T. b. brucei*– and *T. evansi*–infected mice deficient in the p35 gene, the p40 gene, or both, compared with survival among infected wild-type controls. No differences in mortality were observed between the different groups of IL-12–deficient mice.

A specific role for IL-12 in the control of parasitemia in T. b. brucei–*infected mice during the chronic stages of disease.* The first parasitemia peak (occurring 5–8 days after infection, depending on the *Trypanosoma* strain) was similar in all 3 p35- and p40-deficient mice infected with *T. b. brucei* or *T. evansi* and in wild-type controls (figure 1*A* and 1*B*). However, the rate of clearance of the first parasitemia peak was slightly reduced (by 4- to 8-fold) in IL-12–deficient mice, compared with wild-type mice infected with *T. b. brucei,* at day 7 after infection ($P < .05$) (figure 1*A*).

The second parasitemia peak and subsequent parasite control were clearly impaired during *T. b. brucei* infection, with an up to 100-fold increase in the parasite burden occurring in IL-12–deficient mice, compared with wild-type mice. No significant

Table 1. Increased mortality among interleukin (IL)–12–deficient mice infected with *Trypanosoma brucei brucei* or *Trypanosoma evansi,* compared with wild-type mice.

Parasite species,[a] mouse strain	Survival, mean days ± SD	Mice/group,[b] no.	P[c]
T. b. brucei			
Wild type	40.6 ± 8.8	29	
IL-12p35$^{-/-}$/p40$^{-/-}$	35.1 ± 6.4	15	<.05
IL-12p40$^{-/-}$	32.0 ± 3.7	15	<.0001
IL-12p35$^{-/-}$	33.3 ± 4.2	15	<.001
T. evansi			
Wild type	47.0 ± 24.1	33	
IL-12p35$^{-/-}$/p40$^{-/-}$	31.4 ± 14.3	35	<.001
IL-12p40$^{-/-}$	31.1 ± 13.1	21	<.001
IL-12p35$^{-/-}$	30.3 ± 20.6	34	<.01

[a] Mice were intraperitoneally infected with 5×10^3 *T. b. brucei* trypanosomes and 1×10^3 *T. evansi* trypanosomes and were monitored daily for survival.

[b] Results from 3 independent experiments were combined.

[c] Compared with wild-type mice. Statistical significance was determined using the Kaplan-Meier test.

differences in parasitemia were observed between the different IL-12–deficient strains.

In *T. evansi* infection, wild-type mice reduced their parasitemia at days 12, 23, and 36 after infection, forming several waves of parasitemia, as opposed to all IL-12–deficient mice, which failed to reduce their parasitemia (figure 1*B*). No significant differences were observed between the IL-12–deficient strains. In contrast to *T. b. brucei* infection, in which loss of parasitemia control correlated with death among IL-12–deficient mice, *T. evansi* infection was associated with a high parasite burden during infection in both wild-type and IL-12–deficient strains.

Differential regulation of IFN-γ and TNF by IL-12p70, depending on the* Trypanosoma *species. IFN-γ and TNF play a role in resistance and parasitemia control [22, 29], with IL-12 a known promoter of IFN-γ production [30]. Plasma samples were obtained at the first parasitemia peak; at days 6 and 8 after infection with *T. b. brucei* and *T. evansi,* respectively; and also after the second parasitemia peak, at days 20 and 28 after infection with *T. b. brucei* and *T. evansi,* respectively (figure 2). Both *Trypanosoma* species induced systemic IFN-γ and TNF production in wild-type mice, with there being a few days' delay associated with *T. evansi* infection. *T. b. brucei* infection in IL-12–deficient mice resulted in a marked reduction in IFN-γ ($P < .05$) and TNF ($P < .01$) production (figure 2*A* and 2*B*), compared with *T. b. brucei* infection in wild-type mice, with similar residual low levels in all IL-12–deficient strains observed.

In IL-12–deficient mice, *T. evansi* infection also resulted in impaired IFN-γ responses, compared with those seen in wild-type controls, as was also seen in IL-12–deficient mice with *T.*

b. brucei infection (figure 2*C*). In contrast to *T. b. brucei* infection, *T. evansi* infection in IL-12–deficient mouse strains produced substantial amounts of TNF, with only a slight reduction observed (figure 2*D*).

Unimpaired IgG2a and IgM antibody titers in IL-12–deficient mice, compared with wild-type mice, in response to trypanosomiasis. Anti-VSG antibody responses are important in parasitemia control and survival and, thus, were tested in IL-12–deficient mice (figure 3). IgG2a levels were similar between wild-type and IL-12–deficient mice at days 6 (figure 3*A*) and 20 (figure 3*B*) after infection with *T. b. brucei*. At either the first parasitemia peak (figure 3*C*) or during chronic stages of infection (figure 3*D*), IgM levels were similar in IL-12–deficient and wild-type mice infected with *T. evansi.*
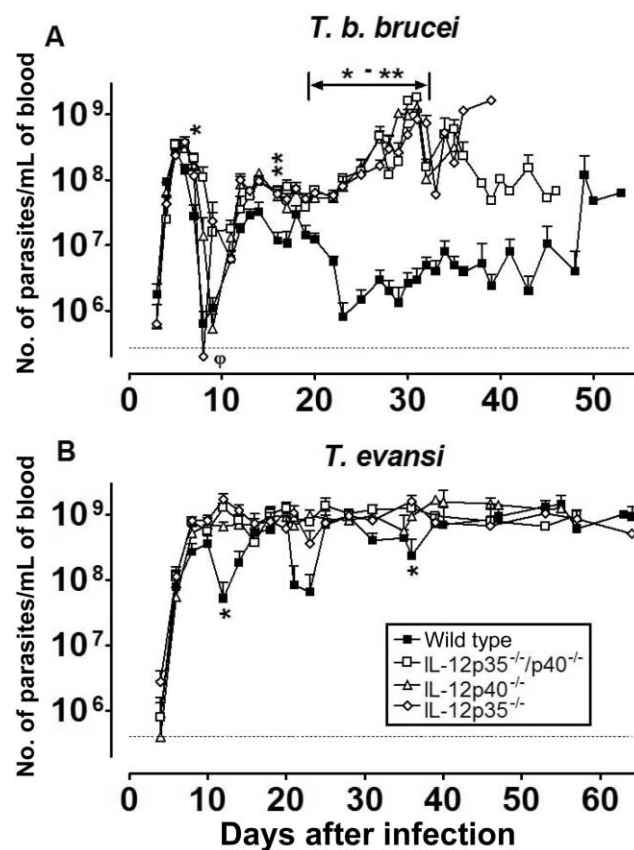

Figure 1. Parasitemia profiles of *Trypanosoma brucei brucei*–infected *(A)* and *Trypanosoma evansi*–infected *(B)* wild-type (■), interleukin (IL)–12p35$^{-/-}$/p40$^{-/-}$ (□), IL-12p40$^{-/-}$ (△), and IL-12p35$^{-/-}$ (◇) mice. Mice were intraperitoneally infected with 5×10^3 *T. b. brucei* trypanosomes and 1×10^3 *T. evansi* trypanosomes, with a lower limit of detection of 2.7×10^5 and 4×10^5 parasites/mL, respectively, for infected mice. Data points are the mean values from 2–3 pooled experiments involving 10–15 individual mice/group. φ, Inferred data points appearing below the limit of detection. The *x*-axis of panel *B* has been cropped from 100 days to 65 days to show detail. At each time point, the statistical significance between infected wild-type and IL-12–deficient mice was determined using 1-way analysis of variance (Dunnett's posttest). *$P < .05$; **$P < .01$.
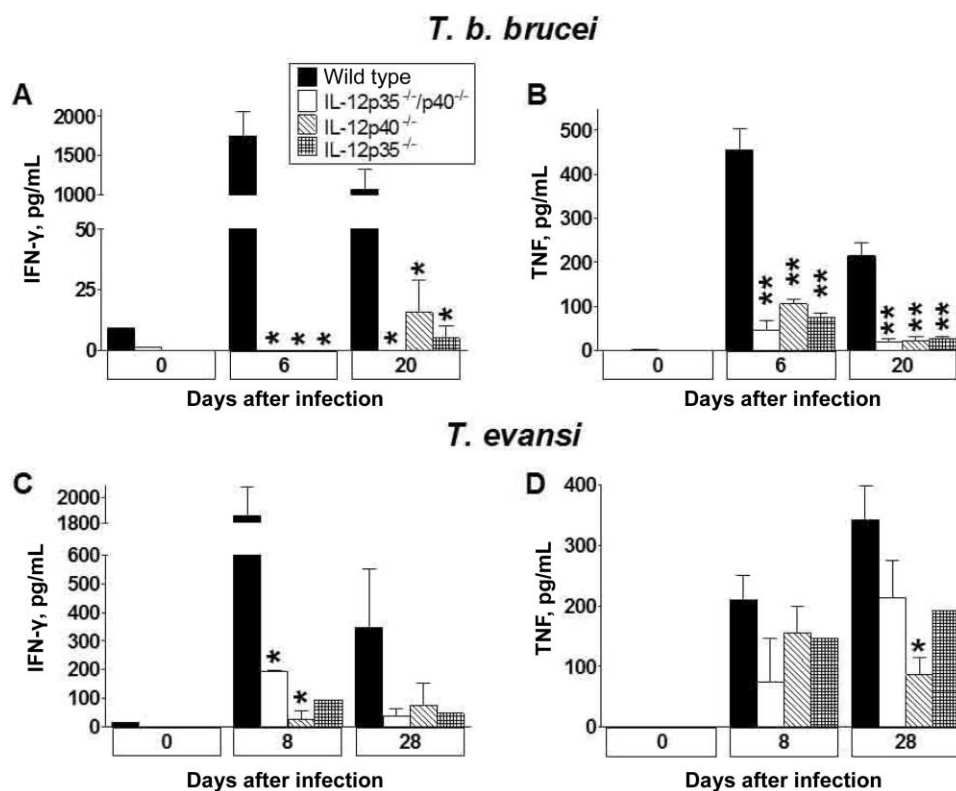
Figure 2. Plasma levels of interferon (IFN)–γ and tumor necrosis factor (TNF) measured in wild-type *(black bars)*, interleukin (IL)–12p35$^{-/-}$/p40$^{-/-}$ *(white bars)*, IL-12p40$^{-/-}$ *(diagonally striped bars)*, and IL-12p35$^{-/-}$ *(hatched bars)* mice infected with *Trypanosoma brucei brucei (A and B)*, and *Trypanosoma evansi (C and D)* by means of ELISA. Results are representative of findings from 2 independent experiments; however, TNF levels in IL-12p40–deficient mice were not consistently significantly reduced. Data are expressed as mean values (±SE) for 2–3 individual mice. Statistical significance was determined using an unpaired Student's *t* test. *P < .05; **P < .01.

IFN-γ production crucial for resistance to trypanosomiasis. Reduced survival was observed among IFN-γR (129Sv/Ev)–deficient mice, compared with wild-type (129Sv/Ev) mice, for both *Trypanosoma* strains, with death occurring at a mean of 16 versus 41 days after *T. b. brucei* infection (P < .001) (figure 4A) and at a mean of 15 versus 27 days after *T. evansi* infection (P < .001) (figure 4C), respectively. IFN-γR–deficient mice infected with *T. b. brucei* were unable to sufficiently clear the first wave of parasitemia and died shortly thereafter (figure 4B), in association with a 10-fold increase in parasitemia from day 9 after infection until death (P < .05), compared with the wild-type controls. The *T. b. brucei* results obtained in this study are similar to those reported for *Trypanosoma brucei rhodesiense*–infected C57BL/6 wild-type and C57BL/6 IFN-γ–deficient mice [22], indicating no major differences between the 129Sv/Ev and C57BL/6 genetic backgrounds with regard to susceptibility due to loss of IFN-γ signalling. Interestingly, *T. evansi* infection resulted in high parasitemia in both wild-type and IFN-γR–deficient strains (figure 4D).

DISCUSSION

IL-12p35$^{-/-}$/p40$^{-/-}$, IL-12p40$^{-/-}$, and IL-12p35$^{-/-}$ mouse strains showed significantly reduced survival, compared with wild-type controls, when infected with either *T. b. brucei* or *T. evansi*. This outcome is in agreement with previous results showing an association with lower IL-12p70 levels in susceptible wild-type mice, compared with mice that were more resistant to trypanosomiasis, during the initial stages of infection [22]. Between the 3 IL-12–deficient mouse strains, no differences in survival rates were observed, suggesting that IL-12p70 is the main protective factor for survival, with there being no major contribution from other IL-12 family members—namely, IL-23 or the IL-12p80 homodimer. IL-12–deficient mice survived several waves of parasitemia; therefore, residual protection occurs and may imply that IL-12 plays a more striking role during established disease.

Because IFN-γ is an important factor in survival, as was previously shown in association with *T. b. brucei* infection [22],
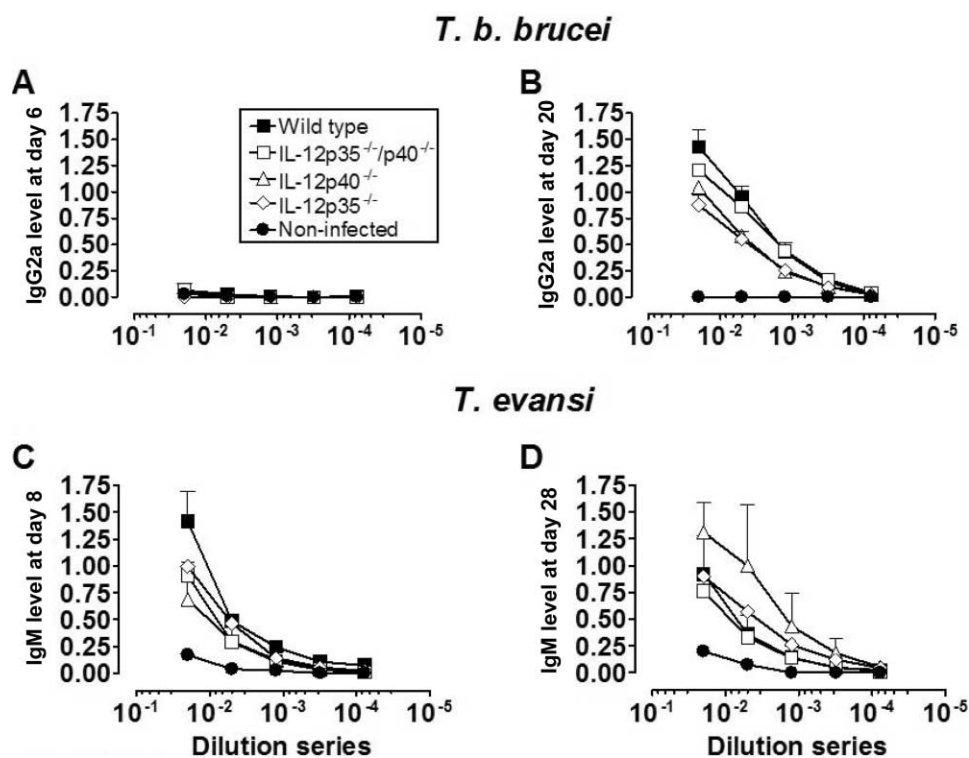
Figure 3. IgG2a and IgM plasma antibody titers of *Trypanosoma brucei brucei*–infected (at day 6 *[A]* and day 20 *[B]* after infection) and *Trypanosoma evansi*–infected (at day 8 *[C]* and day 28 *[D]* after infection) C57BL/6 wild-type (■), C57BL/6 interleukin (IL)–12p35⁻/⁻ (◇), C57BL/6 IL-12p40⁻/⁻ (△), and C57BL/6 IL-12p35⁻/⁻p40⁻/⁻ (□) mice. Preimmune plasma was used as a noninfected control (●). Data points denote mean values (± SE) for plasma antibody titers from 2–3 individual mice.

we determined plasma levels of IFN-γ during infection. In wild-type mice, IFN-γ was present during the first wave of parasitemia, with striking reductions in IFN-γ levels observed in all IL-12–deficient mouse strains. These results also demonstrate that residual IL-12–independent IFN-γ production is induced during infection by both trypanosome strains. Therefore, the observed, limited type-1 response allowed residual protective immunity. IL-12–independent induction of IFN-γ may also include a direct stimulus by either trypanosome species. Trypanosome-derived lymphocyte triggering factor has been found to induce the production of IFN-γ by binding to CD8⁺ cells in *T. b. brucei* [31] and *T. evansi* [32] infections and, therefore, may have contributed to residual resistance in the absence of IL-12. In addition, work describing IL-12–independent IFN-γ production with residual Th1 responses has been demonstrated by us and others during infections with *Listeria monocytogenes* [19], *Mycobacterium* bacille Calmette-Guérin [16], and *Leishmania major* [20]. Furthermore, to evaluate the possible contribution of IFN-γ to resistance, IFN-γR–deficient mice were infected; they died shortly after the first wave of parasitemia. Although the IFN-γR–deficient mice used in the present study were of a different genetic background than the IL-12–deficient mice, the results are in line with the conclusions of Hertz et

al. [22], who studied IFN-γ–deficient mice with a C57BL/6 background. These results clearly highlight the crucial importance of IFN-γ responsiveness during acute infection with *T. b. brucei* or *T. evansi*.

Although IL-12–deficient mice did have significantly reduced rates of parasitemia clearance after the first peak, compared with wild-type mice, the IL-12–deficient mice ultimately were as effective in clearing the first peak as were the control mice. IFN-γ and TNF cytokine levels in all groups of IL-12–deficient mice were significantly attenuated during the initial parasitemia peak. Together with the observation of a drastically reduced capacity to control parasitemia in infected IFN-γR– and TNF-deficient mice [24] during this period, the low plasma concentrations of these 2 cytokines in IL-12–deficient mice are still effective for initial parasitemia control. These results further suggest that IL-12 plays a more dominant role in parasitemia control after the second peak than during the initial parasitemia peak, for optimal promotion of TNF and IFN-γ production.

TNF has been found to have dual effects in *T. b. brucei* infections, such as causing trypanolytic effects [29, 33], reducing the parasite burden in *T. b. brucei* AnTat 1.1 infections [24, 34, 35], and causing immunopathological effects in the host [24]. Although TNF levels were reduced in IL-12–deficient
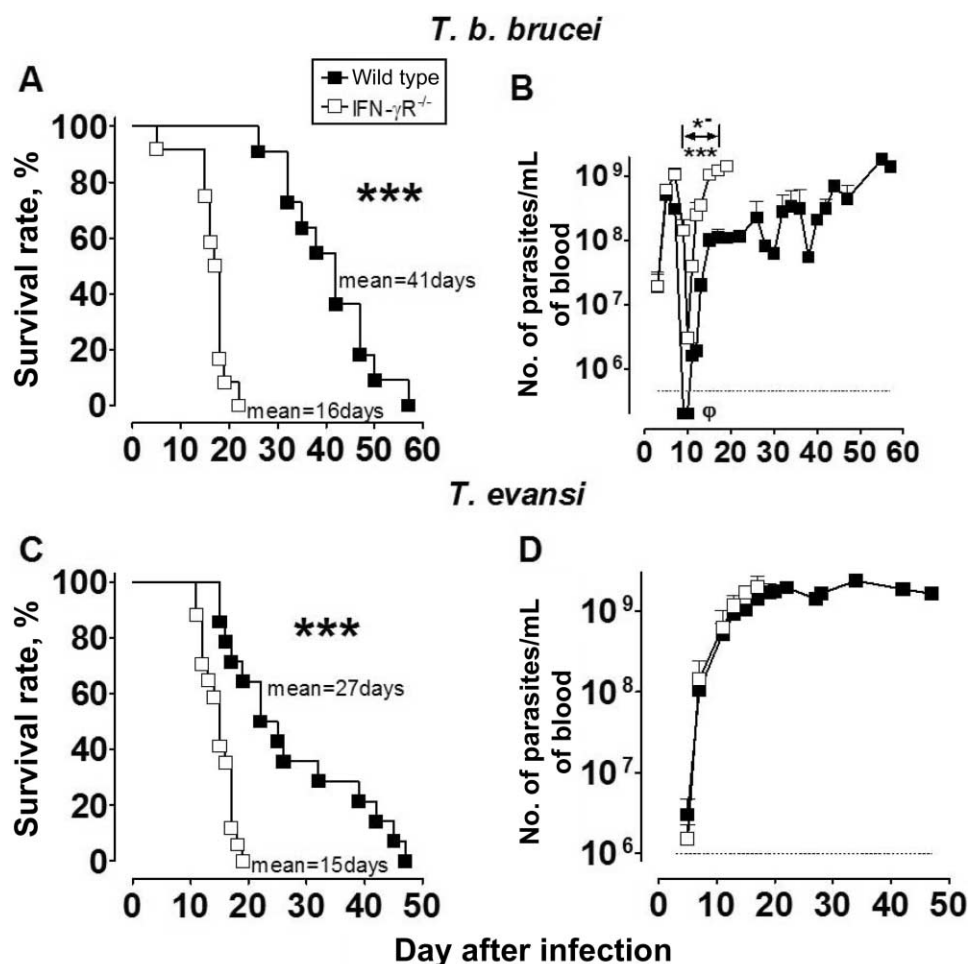
Figure 4. Survival and parasitemia profiles of 129Sv/Ev wild-type (■) and 129Sv/Ev interferon-γ receptor (IFN-γR)–deficient (□) mice infected with *Trypanosoma brucei brucei (A* and *B)* and *Trypanosoma evansi (C* and *D).* Mice were intraperitoneally infected with 5×10^3 *T. b. brucei* trypanosomes (11 wild-type and 12 IFN-γR–deficient mice) and 1×10^3 *T. evansi* trypanosomes (14 wild-type and 17 IFN-γR–deficient mice) per mouse. The limit of detection for *T. b. brucei* parasitemia and *T. evansi* parasitemia is 4.4×10^5 and 1×10^6 parasites/mL, respectively. Data on mortality and parasitemia were pooled from 2 independent experiments performed on 4–5 mice/group used for parasitemia measurement for either experiment. *φ,* Inferred data points appearing below the limit of detection. The Kaplan-Meier test was used for mortality, and the unpaired Student's *t* test was used for parasitemia. *$P < .05$.

mice, no decrease in pathological findings was detected in spleen and liver sections obtained from *T. b. brucei*– or *T. evansi*–infected mice (data not shown). In addition, B cell responses are necessary for the elimination of vascular-bound parasites; in particular, IgM and IgG2a are said to be the main antibodies necessary for clearance of *T. b. brucei* infection (S.M., R.A.A., A. Schwegmann, M. Drennan, F. Claes, P. De Baetselier, F.B., unpublished data) [4]. Both VSG-specific antibodies were present in the plasma of IL-12–deficient mice, albeit in moderately reduced levels, compared with wild-type mice; therefore, they may not be the overall mechanism of increased parasite burden. The role of IL-12 in *T. b. brucei* infection therefore seems to be limited to the more chronic stage of disease and the promotion of the TNF and IFN-γ production necessary to control parasitemia.

Natural killer cells, which are thought to be necessary for resistance during initial infection, are activated by both IL-12 and IL-18 [36], and IL-18 thus may effectively drive the Th1 response in the absence of IL-12. Furthermore, in a recent study, Drennan et al. [37] found that MyD88 signaling is crucial for resistance and that there is a role for Toll-like receptor (TLR) 9 in this immune pathway. The TLR9- and IL-12–deficient mice share many phenotypic characteristics in their response to *T. b. brucei* infection, including (1) effective parasitemia control during the first parasitemia peak but increased parasitemia during the chronic stages of disease and (2) reduced IFN-γ production but normal IgG2a responses during the chronic stages of disease, compared with those noted in wild-type mice. Bone marrow–derived macrophages obtained from TLR9-deficient mice also displayed severely reduced TNF and IL-12p40 levels

in response to *T. b. brucei* genomic DNA. Therefore, IL-12 may be involved in the immunologic pathway, whereby TLR9 present on macrophages responds to *T. b. brucei* genomic DNA released after the first parasitemia peak and, on IL-12 secretion, stimulates peripheral blood mononuclear cells (PBMCs) to secrete IFN-γ. With the presence of TLR9 on B cells, a role for IL-12 may also be considered. However, with only a slight reduction in IgG2a responses during the chronic stages of disease, as reported in this article as well as in the study by Drennan et al. [37], it is more likely that other factors play a more significant role in proliferation and differentiation of these cells. In contrast to IL-12–deficient mice, TLR9-deficient mice produce more IFN-γ during the chronic stages of *T. b. brucei* infection. Reduced IFN-γ production may explain the increased susceptibility in IL-12–deficient mice, compared with TLR9-deficient mice, which showed resistance similar to that noted among wild-type mice. The function of IL-12 may thus be to associate TLR9 signaling in macrophages during the chronic stages of *T. b. brucei* infection with the up-regulation of IFN-γ secretion in PBMCs necessary for effective parasitemia control and survival.

Parasitemia in wild-type mice infected with *T. evansi* differed from that in wild-type mice infected with *T. b. brucei.* Wild-type mice with *T. evansi* infection showed a severe inability to efficiently clear waves of parasitemia, with this inability deteriorating after each successive wave and suggesting a progressive erosion of immune control. IL-12–deficient mice infected with *T. evansi* were unable to reduce parasitemia, compared with wild-type mice. However, later during infection, no differences between IL-12–deficient and wild-type mice were found. This suggests a role for IL-12p70 signaling in early parasitemia control. During the initial and late time points examined, plasma levels of IFN-γ were severely reduced in IL-12–deficient mice, compared with wild-type mice; however, in contrast to *T. b. brucei,* plasma levels were only moderately lower for TNF in IL-12–deficient mice. IFN-γR–deficient mice infected with *T. evansi* were highly susceptible to trypanosomiasis, were unable to clear the first parasitemia peak, and rapidly died thereafter. This finding clearly demonstrates the importance of IFN-γ, although a direct comparison with IL-12–deficient mice is hampered by the difference in the genetic background of the deficient mouse strains and the mortality rate among wild-type controls with *T. evansi* infection. TNF levels were not severely reduced in IL-12–deficient mice and were found not to be important in survival or parasitemia control [38]. In terms of anti-VSG B cell responses, it recently has been shown that IgM is crucial for parasitemia control and survival [38]. However, we found no obvious differences between wild-type and IL-12–deficient mouse strains. Therefore, the mechanism of impaired parasitemia control in *T. evansi* infection is dependent on IL-12, with a possible role for IFN-γ, as has been found in *T. b. brucei* infection.

A comparison between the results obtained by Baral et al. [38] for the C57BL/6 IFN-γ–deficient mice and the results shown in the present article for the 129Sv/Ev IFN-γR–deficient mice reveals opposing findings. This difference could be due to the emergence of a more virulent strain in our experiments, because the *T. evansi* parasites used are nonclonal field stabilates and have been passaged for stock maintenance. In addition, the specific pathogen–free conditions at our facilities may exclude concurrent infections that may independently initiate an IL-12–driven Th1 response necessary for optimal resistance. These effects, although untested, add impetus for further investigation into the immunology of *T. evansi.*

In conclusion, the data presented in this article show that IL-12p70—but not the IL-12p80 homodimer or IL-23—is necessary for optimal resistance to *T. evansi* and *T. b. brucei* infection, in terms of mortality and parasitemia control. The resistance attributed to IL-12 is mediated through IFN-γ, the production of which was significantly decreased in the absence of IL-12. Furthermore, a role for IL-12 in parasitemia control during the chronic stage of *T. b. brucei* infection was shown, in which the presence of IL-12 is needed for optimal TNF and IFN-γ production reducing the parasitemia burden.

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