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Cellular Immunity, but Not Gamma Interferon, Is Essential for Resolution of *Babesia microti* Infection in BALB/c Mice

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A new strain of *Babesia microti* (KR-1) was isolated from a Connecticut resident with babesiosis by hamster inoculation and adapted to C3H/HeJ and BALB/c mice. To examine the relative importance of humoral and cellular immunity for the control of *B. microti* infection, we compared the course of disease in wild-type BALB/c mice with that in BALB/c SCID mice, JHD-null (B-cell-deficient) mice, and T-cell receptor αβ (TCRβ$^{-/-}$) or gamma interferon (IFN-γ) (IFN-γ$^{-/-}$) knockout mice following inoculation with the KR-1-strain. SCID mice and TCRαβ knockouts sustained a severe but nonlethal parasitemia averaging 35 to 45% infected erythrocytes. IFN-γ-deficient mice developed a less severe parasitemia but were able to clear the infection. In contrast, in six of eight JHD-null mice, the levels of parasitemia were indistinguishable from those in the wild-type animals. These data indicate that cellular immunity is critical for the clearance of *B. microti* in BALB/c mice but that disease resolution can occur even in the absence of IFN-γ.

The genus *Babesia* is defined by more than 100 species that collectively infect a wide spectrum of mammals throughout much of the world (11, 14). Maintenance of *Babesia* parasites in nature requires competent vertebrate and nonvertebrate hosts. In the northeastern United States, *Babesia microti* cycles between the white-footed field mouse (*Peromyscus leucopus*) and the deer tick (*Ixodes scapularis*) (11). Most human cases of babesiosis originate from *B. microti* sporozoites that are injected into the bloodstream by infected *I. scapularis* nymphs during feeding. However, some cases have originated from tainted blood transfusions (7). Humans infected with *B. microti* may be asymptomatic carriers, experience flu-like symptoms, or occasionally suffer fatal complications (11, 15).

The widespread recognition of babesiosis as an emerging zoonotic disease (11, 14) has spurred improvements in drug therapies (16) and diagnostics (17, 19, 22), as well as interest in the development of a babesiosis vaccine (11). A better understanding of the immune response to babesial infection could be helpful in designing a safe and efficacious vaccine. While previous reports on mice have shown a strong role for T cells and the Th1 cytokine gamma interferon (IFN-γ) in the control of *B. microti* infection (6, 13, 21), results regarding the role of humoral immunity in parasite clearance have been less clear-cut (2, 4, 13). In this study, we mouse adapted a freshly obtained *B. microti* clinical isolate in order to clarify the contributions of cellular and humoral immunity to disease resolution in a murine model.

Our strain was isolated from a 42-year-old asplenic male afflicted with grade 4 lymphoma who lived in northern Connecticut and worked in an area along the Connecticut coastline where *Babesia* is endemic. He reported a tick bite approximately 1 week prior to the onset of illness. Giemsa stains of the patient's blood at the time of presentation revealed a parasitemia of approximately 5%. Indirect immunofluorescence assay (using the GI strain as antigen) revealed serum immunoglobulin M and G titers of 128 and >1,024, respectively. PCR analysis of whole blood using the Bab1/Bab4 primer pair specific for the *B. microti* 18S rRNA gene (19) yielded a product of the appropriate size (238 bp).

To isolate the strain, subsequently designated KR-1, 1 ml of blood (containing approximately 2×10^8 infected erythrocytes) obtained prior to therapy was injected into a 3-week-old female golden Syrian hamster (*Mesocricetus auratus*). Golden Syrian hamsters are naturally susceptible to infection with *B. microti* and have been used to confirm suspected cases in humans (9). The inoculated hamster was monitored daily by Giemsa staining of tail blood samples. Shortly after the parasitemia peaked at about 1%, the hamster was anesthetized and its blood was harvested by cardiac puncture. Following three additional passages, parasitemias of >30% were obtained. Because golden Syrian hamsters are maintained as outbred colonies, they are not well suited for analysis of the immune processes induced during babesial infection. Consequently, we next adapted the strain to C3H/HeJ mice, which are highly susceptible to infection with *B. microti* (1, 20), by injecting 1 ml of whole hamster blood (approximately 10^8 infected erythrocytes) intraperitoneally into 4- to 6-week-old female C3H/HeJ mice. As before, parasitemia was monitored by PCR analysis and Giemsa staining of tail blood samples. By the sixth passage in C3H/HeJ mice, parasitemias reached 6%.

Although most human babesial infections in the United

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States are due to *B. microti*, in recent years, infections due to piroplasms other than *B. microti* have been reported (14). To characterize the KR-1 isolate, we determined the nucleotide sequence of the Bab1/Bab4 18S rRNA amplicon. The sequence was identical to those of the GI and Ruebush-Peabody strains, both of which were isolated from residents of Nantucket Island, Mass. It was also identical to a sequence obtained from *B. microti*-infested *Ixodes ricinus* ticks from Slovenia (8). The latter finding is in accord with a report stating that allopatric differentiation of *B. microti* strains by the 18S rRNA gene sequence is low (23). In contrast to rRNAs, proteins under immune pressure may reveal higher-resolution phylogenies among strains and/or serve as ecological and pathogenic markers. Indeed, this has been demonstrated by using an immunogenic protein family (BMN1 to -6) identified by serological screening of a *B. microti* expression library (12). The primer pair used for phylogenetic analysis in that study, however, yielded amplicons from only 50% of the samples known to be *B. microti* positive and did not yield a product with our strain.

While the BALB/c background is considered moderately permissive for *B. microti* (1), preliminary experiments revealed relatively low levels of parasitemia following inoculation with 0.5 ml of blood (10^8 infected erythrocytes) from a parasitemic C3H/HeJ mouse. Such low levels of parasitemia, however, were considered to be advantageous for comparison of the course of disease in wild-type animals and animals with various immune deficiencies. Consequently, in a series of experiments, we compared the course of infection in wild-type BALB/c mice with that in BALB/c SCID mice, JHD-null (B-cell-deficient) mice (3), T-cell receptor αβ knockout (TCRβ$^{-/-}$) mice, and IFN-γ knockout (IFN-γ$^{-/-}$) mice. Wild-type, SCID, TCRβ$^{-/-}$, and IFN-γ$^{-/-}$ mice were purchased as breeding pairs from The Jackson Laboratory (Bar Harbor, Maine) and maintained at the University of Connecticut Health Center by one of us (T.V.R.). A JHD-null breeding pair was the generous gift of Mark Shlomchik (Yale University).

Figure 1 presents the parasitemia curves for a comprehensive experiment in which all five groups (each composed of 6-week-old male mice, six mice per group) were compared head to head following inoculation with 200 μl of phosphate-buffered saline-diluted blood (10^6 parasitized red cells) from a heavily parasitemic BALB/c SCID mouse. The *P* values for days 10, 20, 23, 31, 41, and 54 are presented in Table 1. Parasitemia levels in the wild-type controls remained relatively low, peaking at approximately 2% on or about day 20 postinjection. In contrast, SCID mice developed a severe chronic parasitemia that fluctuated around 40%. TCRαβ-deficient mice yielded a parasitemia curve indistinguishable from that of SCID mice; this is consistent with previous studies that have demonstrated a critical role for T cells, particularly CD4$^+$ T cells, in clearing *B. microti* in mice (6, 13, 21). IFN-γ knockout mice consistently developed parasitemias less severe than those of SCID mice yet significantly greater than those of wild-type controls. Interestingly, however, unlike their TCRαβ-deficient counterparts, IFN-γ knockout mice were eventually able to resolve their infections. This result differs from a previous study that reported an inability of IFN-γ-deficient BALB/c mice to clear the Munich strain of *B. microti* (13). Differences in IFN-γ production have been shown in mice

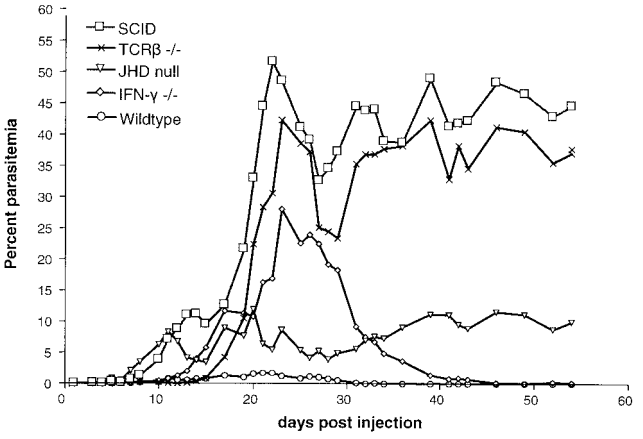

FIG. 1. Parasitemia curves following inoculation of BALB/c wild-type, SCID, TCRβ$^{-/-}$, IFN-γ$^{-/-}$, and JHD-null (B-cell-deficient) mice with 10^6 erythrocytes infected with the KR-1 strain of *B. microti*. Each point represents the average parasitemia of six mice determined from Giemsa-stained tail blood samples. *P* values between experimental groups at selected time points are shown in Table 1.

infected with different *Babesia* species (10). Variation in IFN-γ expression also may occur in mice infected with different substrains of *B. microti* (13, 18). In CBA mice, IFN-γ expression has been shown to increase in the early stages of *B. microti* (King strain) infection (5). Our data indicate that IFN-γ is important for control of the early stages of infection in BALB/c mice but that disease resolution will occur despite a defective Th1 response.

The parasitemia curve for JHD-null mice was not significantly different from that of wild-type controls. However, the JHD mutants displayed two distinct phenotypes. Four mice developed transient parasitemias that quickly resolved to background levels, whereas two mice experienced delayed parasitemias that rose to a chronic level of approximately 30%. In a separate experiment, two additional JHD-null mice developed only transient parasitemias. To ensure that all of the JHD-null mice whose results are depicted in Fig. 1 were deficient in mature B cells, we examined circulating lymphocyte populations by flow cytometry with a panleukocyte panel consisting of goat anti-mouse anti-CD3–fluorescein isothiocyanate, anti-CD19–phycoerythrin–Cy5, and anti-CD45R (eBioscience, San Diego, Calif.). All six JHD-null mice displayed significant reductions in mature B-cell markers in comparison with wild-type controls without considerable variation among staining populations (data not shown).

A dichotomy exists in the literature concerning the role of humoral immunity in resolving *B. microti* infection. Cavacini et al. (2) showed that antibody-depleted BALB/c mice were as resistant as wild-type mice to the King strain of *B. microti*. Additionally, Igarashi et al. (13) were unable to passively protect BALB/c mice with convalescent-phase sera from animals infected with *B. microti* (Munich strain) prior to a homologous challenge. However, Chen et al. (4) demonstrated that convalescent-phase immune serum can inhibit *B. microti* growth in short-term in vitro growth assays. Our results also suggest that antibody is relatively unimportant in the BALB/c background. Because genetic variation among mice can influence *B. microti*-

TABLE 1. *P* values[a] for parasitemia curves at selected time points postinoculation

Comparison	*P* value					
	Day 10	Day 20	Day 23	Day 31	Day 41	Day 54
SCID vs T Δ[b]	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
SCID vs B Δ[c]	>0.05	>0.05	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SCID vs IFN-γ Δ[d]	>0.05	>0.05	**<0.05**	**<0.001**	**<0.001**	**<0.001**
SCID vs +/+[e]	>0.05	**<0.05**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TΔ vs BΔ	>0.05	>0.05	**<0.001**	**<0.001**	**<0.01**	**<0.001**
TΔ vs IFN-γ Δ	>0.05	>0.05	>0.05	**<0.001**	**<0.001**	**<0.001**
TΔ vs +/+	>0.05	>0.05	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BΔ vs IFN-γΔ	>0.05	>0.05	**<0.05**	>0.05	>0.05	>0.05
BΔ vs +/+	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
IFN-γ Δ vs +/+	>0.05	>0.05	**<0.001**	>0.05	>0.05	>0.05

[a] *P* values were derived by one-way analysis of variance and Tukey's multiple-comparison test. Significant *P* values (< 0.05) are in bold.
[b] TΔ, TCRβ$^{-/-}$ mice.
[c] BΔ, JHD null mice.
[d] IFN-γΔ, IFN-γ$^{-/-}$ mice.
[e] +/+, wild-type BALB/c mice.

host interactions considerably (1), the role for humoral immunity in the resolution of babesiosis needs to be examined in other genetic backgrounds. Moreover, the finding that humoral immunity is unnecessary for clearance of an established infection does not necessarily preclude a role for antibodies in protection against tick transmission of *B. microti* sporozoites, the pre-erythrocytic form of the parasite that is likely to display antibody-accessible antigens. The mouse-adapted KR-1 strain used for the needle inoculation studies reported herein is infectious throughout the tick-mouse cycle (unpublished observations) and, thus, can be used to assess this notion.

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