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Infection of severe combined immunodeficient mice with Babesia sp. strain WA1 was studied to assess the contributions of innate and adaptive immunity in resistance to acute babesiosis. The scid mutation showed little effect in genetically susceptible C3H mice and did not decrease the inherent resistance of C57BL/6 mice to the infection, suggesting that innate immunity plays a central role in determining the course of Babesia infection in these strains. In contrast, the scid mutation dramatically impaired resistance in moderately susceptible BALB/c mice, suggesting that acquired immunity may play an important secondary role. In comparison to their female counterparts, male mice of different genetic backgrounds showed increased resistance to the infection, indicating that the gender of the host may influence protection against babesiosis.

Babesia infection results in a wide array of disease manifestations, ranging from an asymptomatic carrier state to malaria-like episodes that are often life threatening. Studies with inbred mouse strains have identified a genetic influence on the course of infection: C57BL/6 mice are highly resistant to babesiosis, in contrast to the susceptibility demonstrated in A/J, C3H, 129, and BALB/c strains. This effect is independent of the major histocompatibility complex and attributable instead to other genes in the C57BL genetic background. This background effect has been observed for both Babesia microti (9, 24) and Babesia sp. strain WA1 (21), a recently discovered species isolated from a human patient (22, 28).

The role of the immune response in resistance to babesiosis has been studied extensively (16, 30), but a clear picture of the roles of specific immune components has yet to emerge. In chronic infections with B. microti, T lymphocytes, specifically those in the subpopulation of gamma interferon (IFN-γ)-producing CD4+ T cells, seem to play a role in parasite clearance (7, 17, 20, 25). Immunodepletion experiments have shown that CD4+ T cells protect against infection with B. microti. However, these cells are not protective against lethal Babesia rodhaini infection (26).

Severe combined immunodeficient (SCID) mice carry a mutation that produces an abnormal recombinase system in developing lymphocytes, resulting in the absence of functional T and B cells. Still, SCID mice exhibit normally functioning myeloid, antigen-presenting, and natural killer cells (8). In the present study, we infected mice carrying the scid mutation on Babesia-resistant and -susceptible genetic backgrounds to assess the relative roles of innate and acquired immune responses in resistance to infection with the WA1 strain of Babesia.

Five- to 7-week-old mice from the C57BL/6J-Prkdcscid/SzJ, C3HSmn.C-Prkdcscid/J, and BALB/cByJSmn-Prkdcscid/J strains (B6 SCID, C3H SCID, and BALB/c SCID, respectively) as well as inbred C57BL/6, C3H/HeJ, BALB/c, AKR, and 129/J mice (Jackson Laboratory, Bar Harbor, Maine) were infected intraperitoneally with Babesia sp. strain WA1 as described previously (21). Parasites were maintained by cryopreservation in liquid nitrogen, and pathogenicity was confirmed by passage in female Syrian golden hamsters. The same inoculum was used to infect all mice included in an experiment, each receiving 107 parasitized erythrocytes in 100 μl of saline.

Infected mice were monitored daily for mortality. Peripheral blood from four to seven mice was sampled every 3 to 4 days. Parasitemia was determined by microscopic examination of Giemsa-stained thin blood smears. Survival was analyzed by the log rank and Wilcoxon tests using the Kaplan-Meier non-parametric model. Parasitemia was evaluated by mixed-model analysis of variance as implemented in PROC MIXED (SAS) analysis software. Data were analyzed on the square root scale in order to meet modeling assumptions. Mean parasitemia levels and 95% confidence intervals were computed from the data for all mice in the groups after adjusting for repeated measurements.

In order to determine the extent to which innate immunity affects resistance to the strain WA1 parasite, male mice carrying the scid mutation on susceptible (C3H), intermediate (BALB/c), and resistant (C57BL/6) genetic backgrounds were infected with the WA1 strain of Babesia. Immunocompetent counterparts of the three inbred strains were used as controls. The C3H and C3H SCID mice sustained similar courses of acute babesiosis, with marked weakness, tachypnea, hypothermia, piloerection, and hematuria, resulting in an invariably fatal outcome (Fig. 1A). The disease was accompanied by a substantial increase in parasitemia, which peaked between days 10 and 12 after inoculation and reached up to 45% of circulating erythrocytes (Fig. 1B). Interestingly, the rate of survival of the C3H SCID mice was significantly higher than that of the C3H controls (Fig. 1A), which suggests that T and/or B cells may play a pathological role in susceptible animals. In contrast, infection with strain WA1 in the C57BL/6 mice resulted in no signs of disease and a survival rate of 100% (Fig. 1A). Parasitemia reached a maximum of 5% around day 12 postinoculation, rapidly decreasing afterwards to undetect-
able levels (Fig. 1B). The presence of the scid mutation in the C57BL/6 genetic background did not affect the survival (Fig. 1A) or the parasitemia levels (Fig. 1B) of the mice during the initial course of infection. A moderate increase in parasitemia in the B6 SCID mice was found after day 20 (Fig. 1B), suggesting that adaptive immune cells may be involved in protection from recurrent parasitemia.

The moderately susceptible BALB/c mice developed mild signs of infection and displayed a mortality rate of about 10% (Fig. 2A). Parasitemia peaked around day 14 after challenge, with levels that were higher than those of the B6 mice (P = 0.003), and resolved shortly afterwards (Fig. 2B). However, BALB/c SCID mice underwent a severe infection course, which eventually led to a fatal outcome in all of the infected animals (Fig. 2A). Parasitemia in BALB/c SCID mice was significantly increased compared to that of BALB/c controls as well as to that of SCID mice with a B6 genetic background, reaching up to 25% of total circulating erythrocytes (Fig. 2B).

Thus, BALB/c mice exhibit partial resistance to the parasite, but such resistance is dramatically compromised by the scid mutation, suggesting that adaptive immune cells are involved in the protective response in this strain and that innate immu-
Innate immunity alone is insufficient for protection against acute babesiosis in moderately susceptible hosts. Taken together, these observations suggest that resistance to acute babesiosis with Babesia sp. strain WA1 can largely be achieved by innate immunity and that, in contrast to the B. microti model (17), resistance does not seem to require IFN-γ-producing CD4⁺ T cells. Innate immunity may be sufficient for protection against acute babesiosis, at least in the context of the resistant C57BL/6 genetic background.

The contrast between resistant B6 SCID and susceptible BALB/c SCID mice suggests that genetic differences between these strains may manifest themselves in the ability to control Babesia infection by innate immunity mechanisms. It is known that the BALB/c strain carries genetic determinants that influence immune responses and that are associated with susceptibility to intracellular pathogens (11). These host determinants are likely to affect the nature of the innate immune responses in BALB/c SCID mice. Specifically, it is possible that the genetic differences in the interleukin-12 responsiveness between C57BL/6 and BALB/c mice previously described (13, 14) could be the basis for these findings.

In the C3H mouse, not even the concerted actions of innate and acquired immune responses were able to provide protection against the parasite and the disease process that it triggers. Additional genetic polymorphisms in this strain could be responsible for its high susceptibility, possibly causing an exaggerated inflammatory response to infectious stimuli that contributes to the pathological process (5, 21).

No differences in susceptibility to the WA1 strain of Babesia were found between males and females in either the resistant C57BL/6 or the susceptible C3H/HeJ and BALB/c strains (data not shown). However, increased resistance in males was noticed with mice from 129/J and AKR inbred strains as well as in (B6 × 129)F₁ hybrids and the progeny of a (C3B6 × C3H)F₁ backcross (Fig. 3). The magnitude of the gender-related differences in resistance differed among the groups, but males were always significantly more resistant than females. These data suggest a gender influence in mice in resistance to acute babesiosis.

To determine whether the observed gender-dependent difference in resistance was associated with innate immune responses, C57BL/6 SCID mice were challenged with Babesia sp. strain WA1 and the severity of the infection was analyzed relative to gender. We found that male mice were significantly more resistant than females, as indicated by survival rates and parasitemia levels (Fig. 4), which is consistent with the observation made for genetically diverse strains indicated in Fig. 3.

The finding of gender-related differences in the resistance of the B6 SCID mice to Babesia infection could imply that gender-related factors might act at least in part through their effects on innate immunity. Furthermore, the fact that these differences could not be seen in the B6 control mice may indicate that gender-related influences are relatively subtle and thus are revealed only in suboptimal immunogenetic contexts.

Our data provide direct evidence that in the absence of T and B cells, infected C57BL/6 mice can mount a protective response against Babesia, which supports the view that innate mechanisms play a prominent role in immunity to babesiosis (6, 16, 30). Observations of BALB/c SCID mice, however, suggest that acquired immunity may still play an important role if the genetic context decreases the efficiency of innate immune responses.

Innate immune responses probably play a critical role in determining the level of parasitemia at early stages of infection. Protection against other protozoan parasites in the absence of B and T lymphocytes has been observed in other systems; e.g., SCID mice exhibit partial resistance to Toxoplasma gondii (29), Leishmania major (19), Listeria monocytogenes, and Cryptosporidium parvum (3).

Our data also show a gender-related influence in resistance to acute babesiosis in mice, with males being more resistant than females. Gender-dependent differences in susceptibility to other parasitic infections have been extensively documented (1). Females are more resistant to Plasmodium chabaudi (27), Leishmania mexicana (2), Trypanosoma cruzi (15), Trypanosoma rhodesiense (12), and Giardia muris (10) infections, whereas males are more resistant to Leishmania major (2, 4) and Toxoplasma gondii (18) infections. In the Toxoplasma gondii model, gender-dependent differences in cytokine production which correlate with resistance to the parasite mediated by innate immune responses have been described (23, 29). Our findings in B6 mice suggest that gender-related factors may influence resistance through its effects on innate immunity mechanisms.

In summary, our studies show that innate immunity can play a substantial role in the resistance to Babesia infection and that genetic and gender-related factors influence the efficiency of the protective response.
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REFERENCES


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