

B cells and CD4+ T cells play a key role in resistance to *Babesia microti* infection in mice

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Babesiosis is an emerging disease caused by the intraerythrocytic parasite *Babesia microti*. In immunocompetent individuals, *B. microti* infection is typically asymptomatic or appears as a mild flu-like disease that quickly resolves itself. Immunocompromised patients, particularly those treated with rituximab or suffered from immunodeficiency, experience severe, persistent and relapsing babesiosis. Understanding the mechanisms of the B-cell and $CD4^+$ T-cell-dependent immune response responsible for controlling *B. microti* infection, and affecting the disease outcome, is essential for explaining the primary cause of babesiosis relapse and the development of optimal treatment for immunocompromised patients infected with *B. microti*.

Male C57Bl/6 mice were inoculated intraperitoneally with *B. microti*-infected erythrocytes. Parasitaemia was assessed by examination of Diff-Quick-stained blood smears. *B. microti*-infected mice were treated with intraperitoneal injection of anti-CD20 or/and anti-CD4 mAbs to deplete $CD20^+$ B cells or/and $CD4^+$ T cells, and exogenous IL-10. The frequencies of regulatory B and *T* cells were evaluated by flow cytometry. IgM and IgG were quantified by ELISA using *B. microti*-infected erythrocytes as a source of parasite antigens. Serum cytokine levels were measured using ELISA.

Depletion of B cells or/and $CD4^+$ T cells resulted in susceptibility to *B. microti* infection in mice. Mice depleted of B cells developed the highest level of parasitaemia during second peak compared to all experimental mice. Mice treated with both anti-CD20 and anti-CD4 exhibited a lower increase in parasitaemia, but a high level of parasitaemia persisted until the mice were sacrificed, in contrast to B-cell or $CD4^+$ T-cell depleted mice. Mice depleted of B cells showed a significantly reduced frequency of regulatory T cells in the spleen following *B. microti* infection in relation to control mice. Simultaneous anti-CD20 and anti-CD4 treatment resulted in a decreased serum level of IgM and IgG compared to the control mice. Moreover, $CD4^+$ T-cell-depleted mice also exhibited lower concentrations of IgM and IgG than control mice. The serum level of IFN-γ was significantly declined in mice treated with anti-CD20 alone and with anti-CD4.

Our results confirm that B cells and $CD4^+$ T cells are critical for resistance to *B. microti* infection in C57Bl/6 mice and, through production of IFN-γ and antigen-specific IgM and IgG, mediate a crucial part of immune response that protects the host from parasite infection.