An immunological atlas of Babesia microti primary infection and reinfection immunity in Balb/c mice

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Abstract

Correlates of protective immunity to and pathogenesis of Human Babesiosis have not been clearly defined. *Babesia microti* (the most common cause of Human Babesiosis) causes an acute but self-resolving infection in Balb/c mice. We developed an immunological atlas of *B. microti* infection in Balb/c mice over the course of a primary infection and reinfection by performing histological analysis of multiple organs, in depth flow cytometry, and immune profiling of antibody isotypes and cytokines in serum. Histopathologic observations included hepatic vascular congestion, pulmonary vascular erythrocyte margination, clumping, and intrahistiocytic cytoplasmic parasite adherence to the endothelium. By flow cytometry, we report 4 major findings. First, B. microti infection of Balb/c mice is associated with expansion of a novel population of $CD4^-CD8^-$ T cells. Second, *B. microti* induces a potent Th2 $CD4^+$ T cell response characterized by 3.6 fold more Th2 associated $GATA\text{-}3^+CD4^+$ T cells compared to Th1 associated $T\text{-}bet^+CD4^+$ T cells on day 3 post-infection ($p<0.01$, Mann–Whitney U test). Third, naïve $CD4^+$ T cells differentiate into short lived $CD62L^-CD127^-$ effector cells during primary infection. Fourth, B cells undergo isotype switching and differentiate into $CD38^+CD138^+$ plasmablasts and $CD95^+GL7^+$ germinal center B cells. Among 23 cytokines analyzed, IL-13 was the most abundant cytokine produced during Acute Babesiosis in Balb/c mice. These results identify novel immune cells and cytokines associated with the acute and clearance phases of B. microti infection in a mouse model of Human Babesiosis.

Introduction

There is no vaccine against Human Babesiosis and currently available drugs are less effective to treat immunocompromised patients sometimes resulting in mortality. Our lab is working to identify host biomarkers that could serve as novel vaccine and therapeutic targets to ameliorate the most severe symptoms of babesiosis. We are utilizing a murine model of Babesia microti to study immunity and pathogenesis of Babesiosis. We present results of immunological studies (histological, flow cytometry, and cytokine analysis) performed in *B. microti* infected BALB/C mice.

Materials and Methods

Infection of mice: Balb/c mice were infected by intravenous infection with 10^3 *Babesia microti* parasites. Thin blood films were prepared every other day beginning on day 2 post-infection and parasitemia (parasitized erythrocytes, total erythrocytes x 100) was enumerated by examination of Giesma-stained thin blood films.

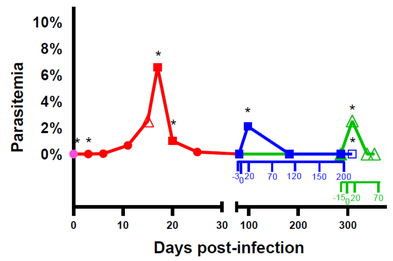
Detection of serum cytokines: Serum levels of 23 cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF–α) was measured using the Bioplex Pro Mouse Cytokine 23-plex assay (Bio-Rad Laboratories, Hercules, CA).

Flow Cytometry: Flow cytometric analysis was performed on immune cell subsets in spleen tissue in naïve mice and *B. microti* infected mice on days 3, 17, and 20 post-infection. Single cell suspensions were labeled with viability dye eFluor™ 506 to distinguish live from dead cells, incubated with TruStain FcX™ to block Fc receptors, and stained with a panel of antibodies appropriately titrated antibodies. Events Were then acquired on a four lasor LSRFortessa X-20™ flow cytometer.

Results and Discussion

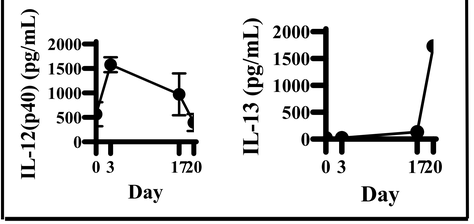
Results 1

- ***B. microti* infection and re-infection in Balb/c mice**



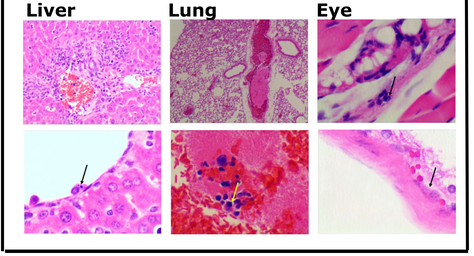
Results 2

- **Mice produce abundant levels of IL-12(p40) during the early phase of infection and IL-13 during the clearance phase of infection**



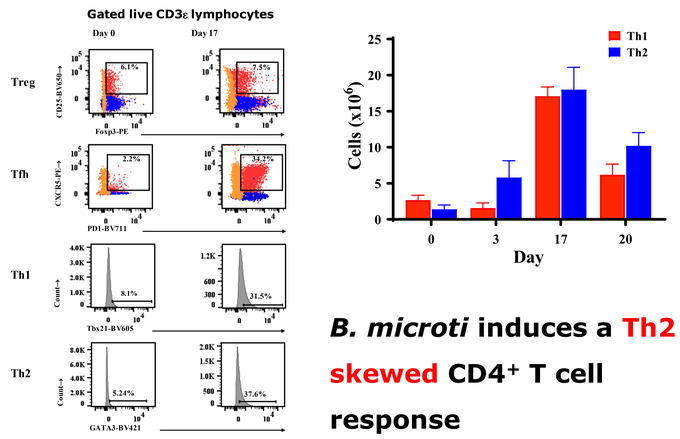
Results 3

- **Histology of *B. microti* infection in Balb/c mice**



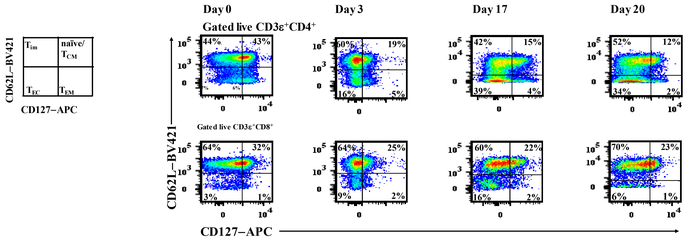
RESULTS 4

- **Effect of *B. microti* on CD4 T cell differentiation**



***B. microti* induces a Th2 skewed $CD4^+$ T cell response**

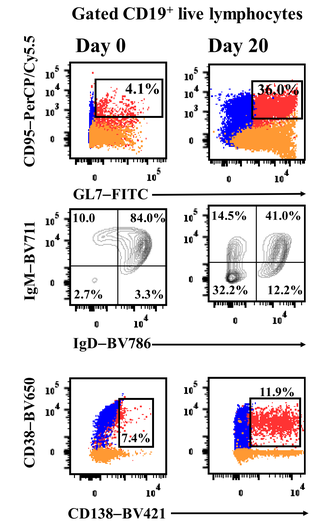
RESULTS 5



Naïve $CD4^+$ T cells differentiate into short-lived $CD62L^-CD127^-$ effector cells during primary infection

Results 6

***B. microti* infection induces differentiation into activated germinal center B cells, isotype switched B cells, and plasma cells**



Conclusion

We performed in depth analysis on the immune response to *B. microti* infection in Balb/c mice. During primary infection, parasitemia peaked at approximately 6%. Partial but incomplete immunity was observed during two secondary infections given at different time intervals. During primary infection, IL-12(p40) was produced during the early phase of infection and IL-13 was produced during the clearance phase of infection. B. microti infection induced a Th2 skewed $CD4^+$ T cell response and naïve CD4+ T cells differentiated into short-lived $CD62L^-CD127^-$ effector cells. B microti infection also induced differentiation of naïve B cells into activated germinal center isotype switched B cells and into $CD138^+CD38^+$ plasma cells.