

# Chronic *Trypanosoma cruzi* infection in mice lacking B cells (muMT)

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## Abstract

**Introduction:** B cells are crucial players during the acute *T. cruzi* infection. However, very little is known about their role in the chronic phase of the disease. This study analyses the consequences of the B cell absence in C57BL/6 mice infected with low numbers of the Tulahuen *T. cruzi* strain. **Material and Methods:** C57BL/6 or C57BL/6 muMT mice were infected with the Tulahuen *T. cruzi* strain (50 blood trypomastigotes, *i.p.*). Mice that survived the acute challenge were used between 4 and 8 months after infection. The following experiments were carried out: 1- Spleen and mononuclear heart and striated muscle cells were studied by flow cytometry. 2- Cytokines were measured by ELISA in supernatants from spleen cells after *in vitro* cultures. Histopathological studies of the heart and muscle were performed. **Results:** Despite very high parasitemia and high mortality, muMT mice controlled the number of parasites in circulation. When infected with low parasite numbers, about 30% of the mice survived and progressed to the chronic phase of the disease. Four to eight months after the initial challenge, mice started to die due to infection re-aggravation. MuMT mice that succumbed to the chronic infection were all lymphopenic, and this picture was never observed in B-cell sufficient mice (Figure 1A and 1B). Spleen cells from muMT chronically infected mice produced equivalent amounts of IL-4 and IL-10 than wt mice (Figures 2A and 2B) but lower amounts of IL-2, INF- $\gamma$  and IL-12 (Figures 3A, 3B and 4A). However, the production of IL-18 was much higher than wt mice (Figure 4B). Histopathological studies showed a more intense inflammatory infiltrate in the striate muscle of muMT chronically infected mice. **Conclusion:** The results presented in the present study demonstrate that B cells are crucial to maintaining T cell numbers and functions during the chronic phase of *T. cruzi* infection, regulating tissue inflammation.

## Results

### Results

#### 1- Splenic T cell numbers

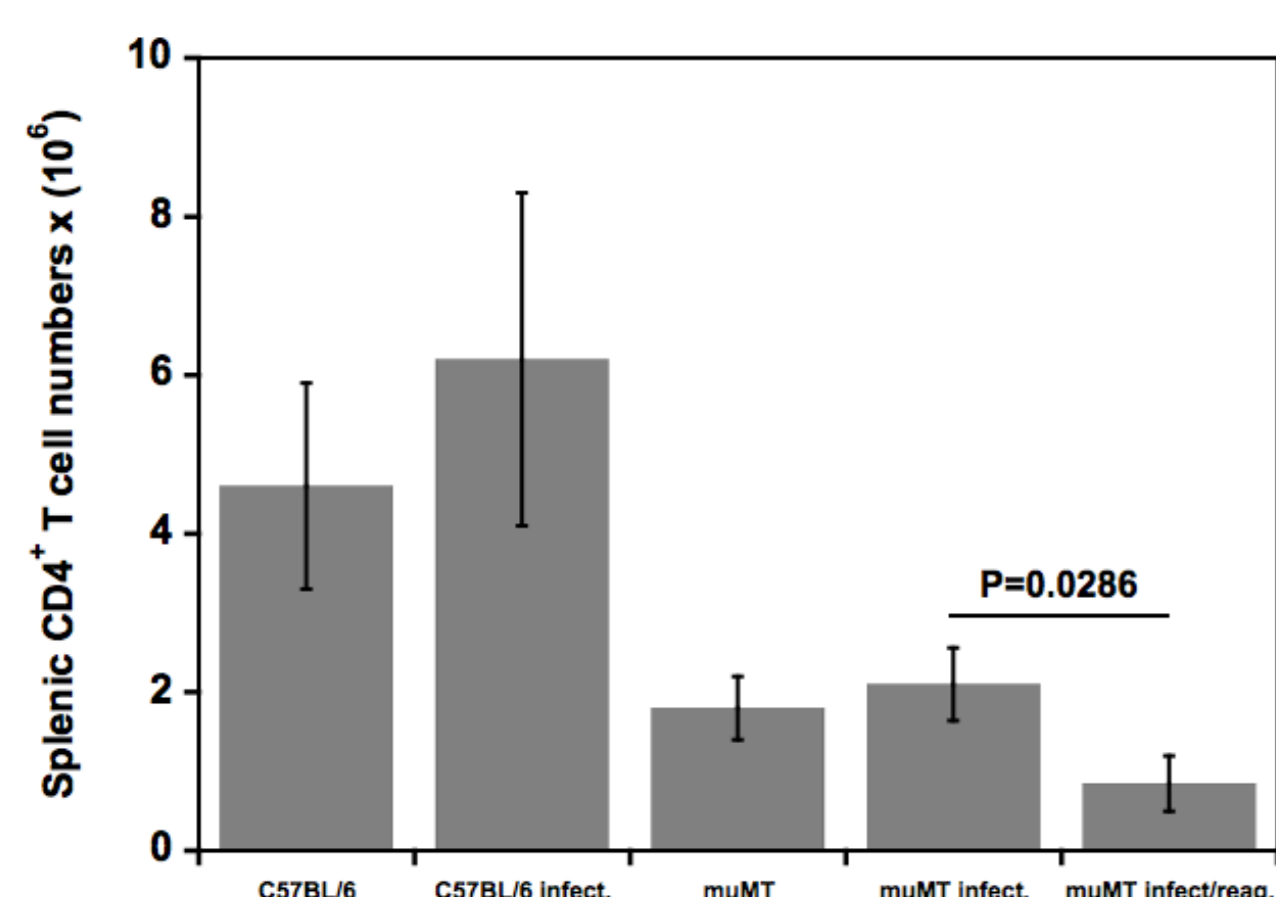


Figure 1A. The number of splenic CD4<sup>+</sup> T cells in different experimental groups (X-axis). Results were obtained by analyzing cytometry data. Two groups muMT infect. (mice were in the chronic stage of the infection) and muMT infect/reag (mice in the chronic phase of the disease, with parasitemia re-aggravation). The Mann-Whitney test, N=4, compared groups.

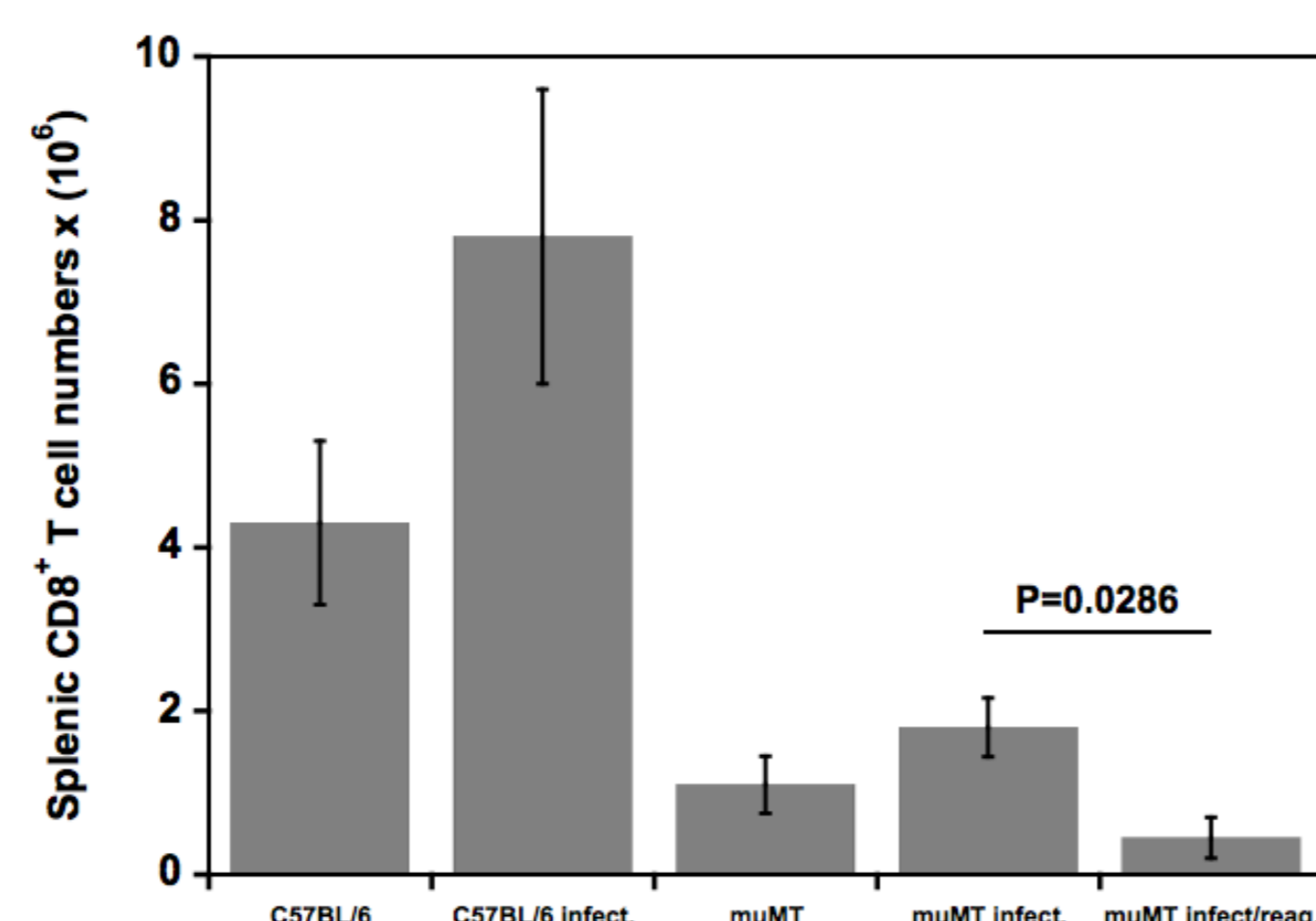


Figure 1B. The number of splenic CD8<sup>+</sup> T cells in different experimental groups (X-axis). Results were obtained by analyzing cytometry data. Two groups muMT infect. (mice were in the chronic stage of the infection) and muMT infect/reag (mice in the chronic phase of the disease, with parasitemia re-aggravation). The Mann-Whitney test, N=4, compared groups.

#### 2- The production of IL-4 and IL-10 by spleen cells

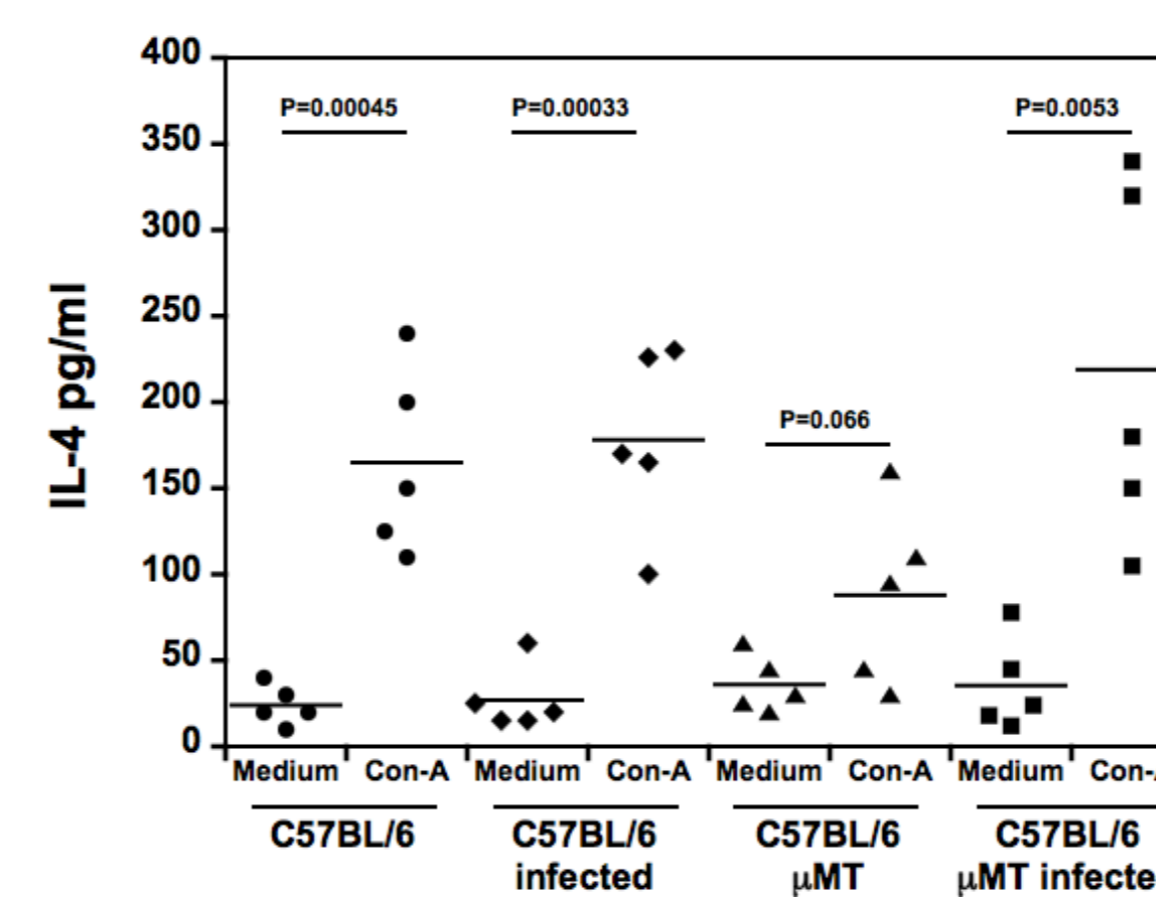
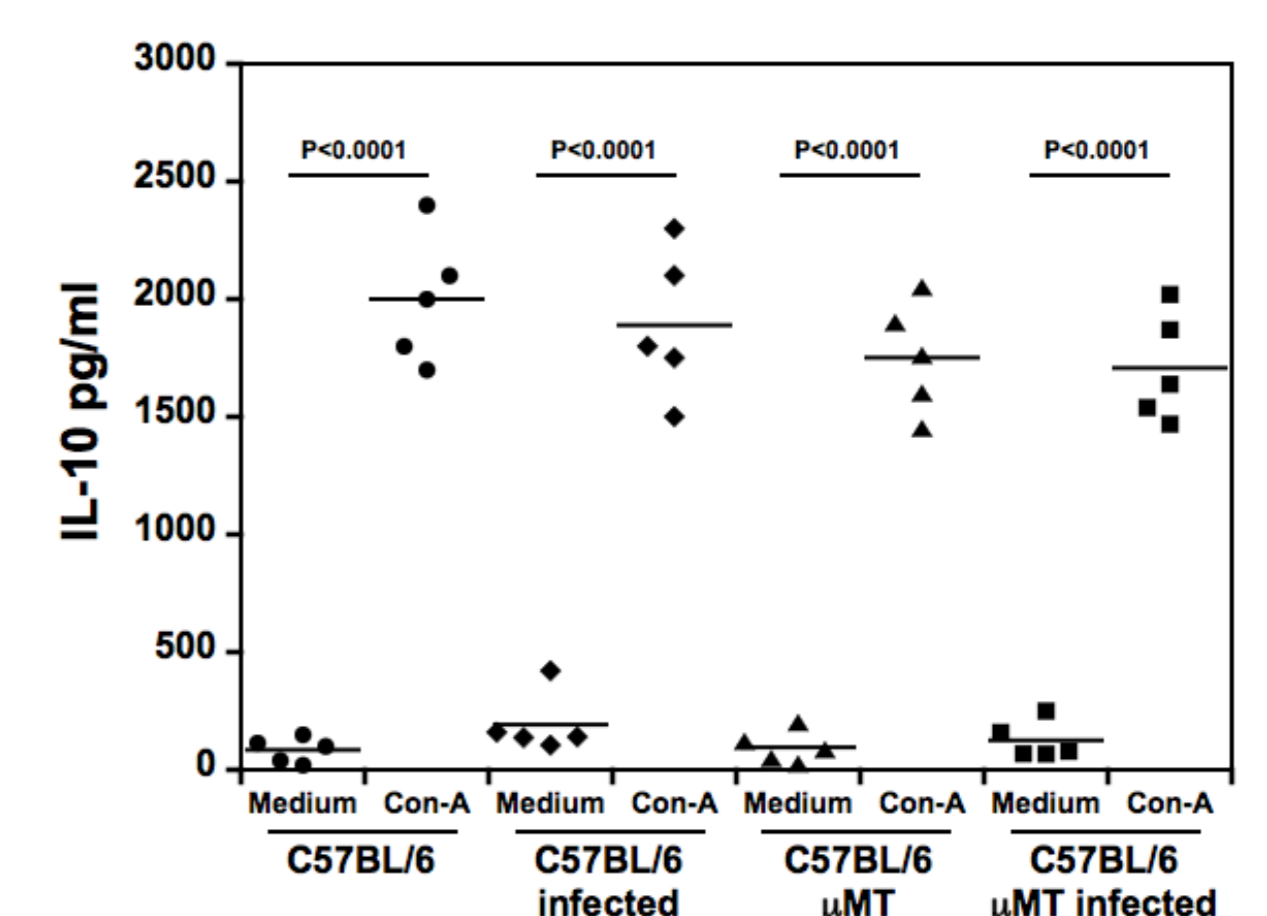


Figure 2A. The production of IL-4 was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.

Figure 2B. The production of IL-10 was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.



#### 3- The production of IL-2 and IFN-gamma by spleen cells

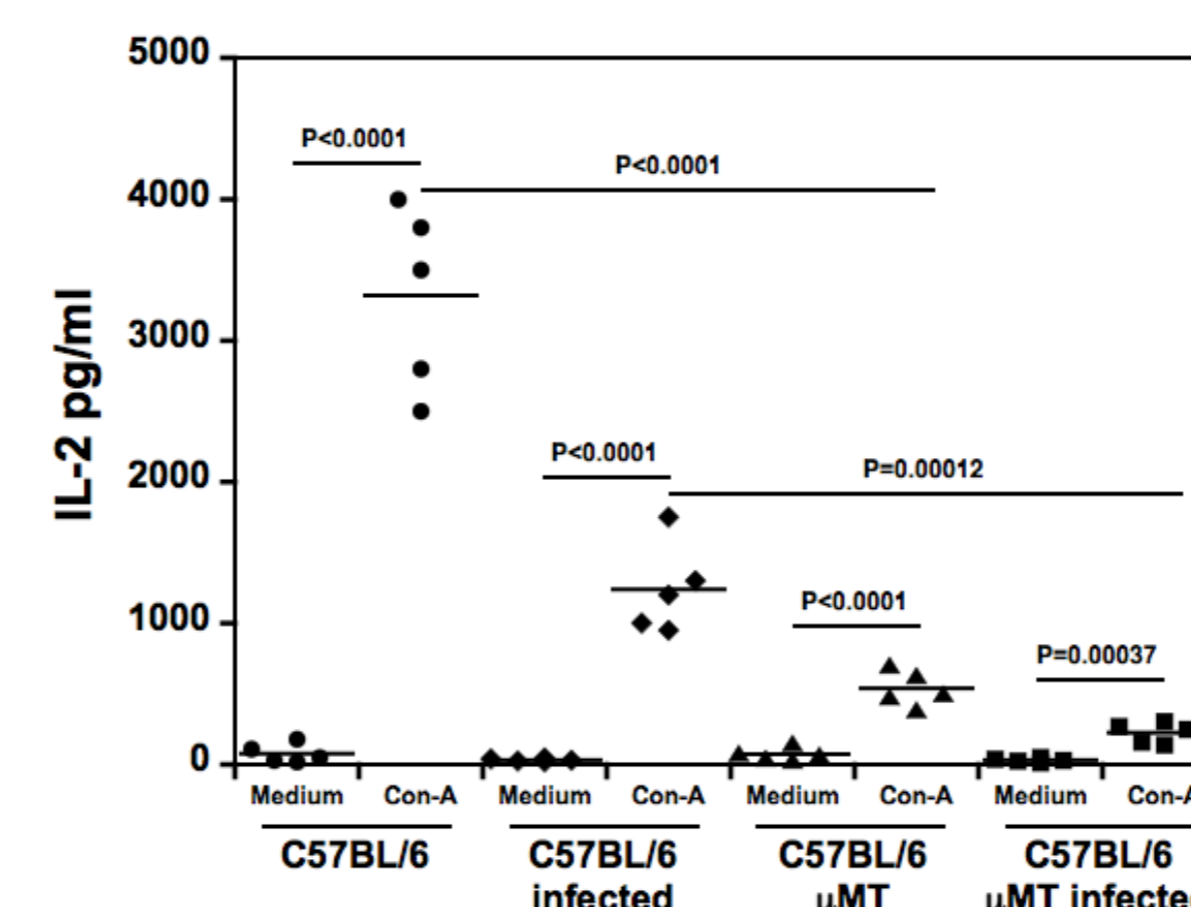


Figure 3B. The production of IFN- $\gamma$  was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.

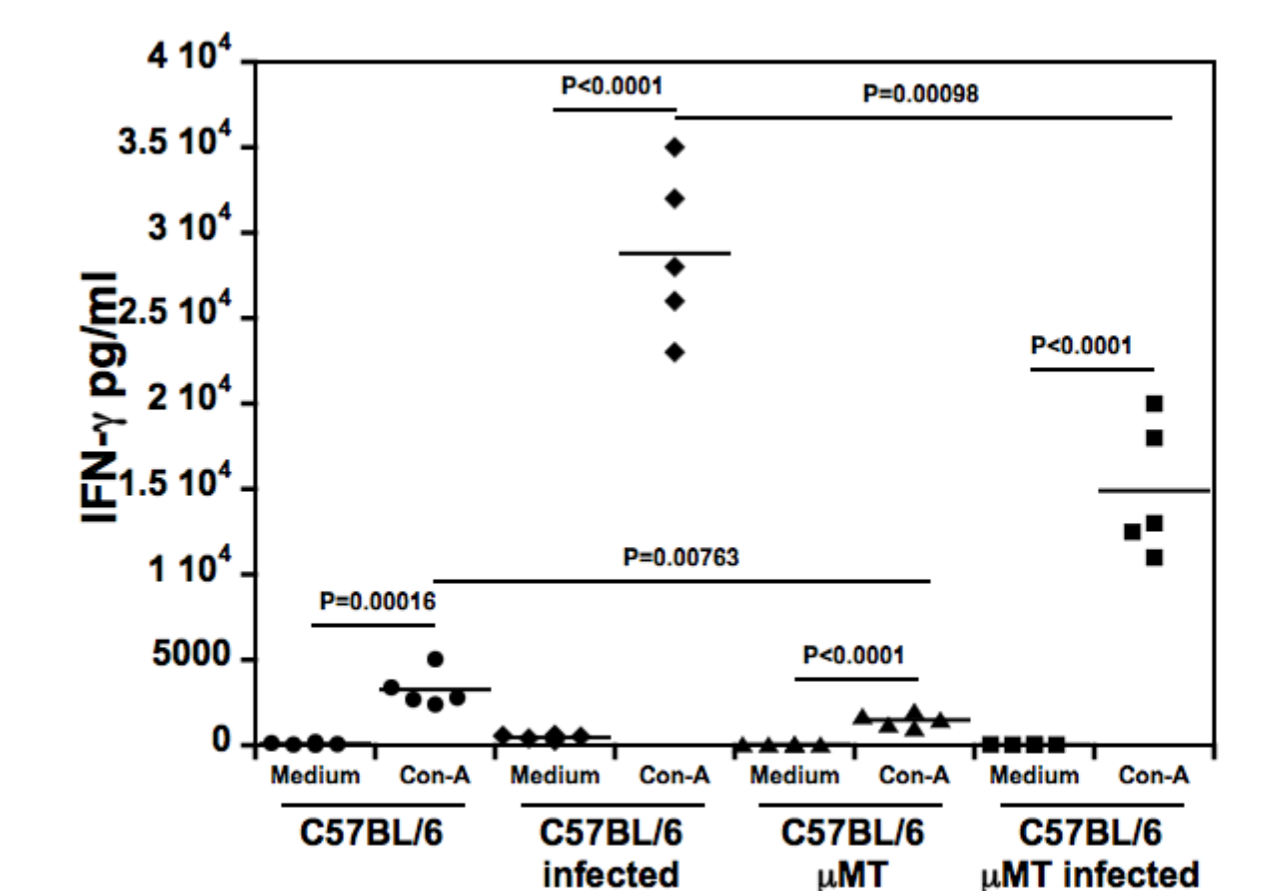


Figure 3A. The production of IL-2 was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.

#### 4- The production of IL-12 and IL-18 by spleen cells

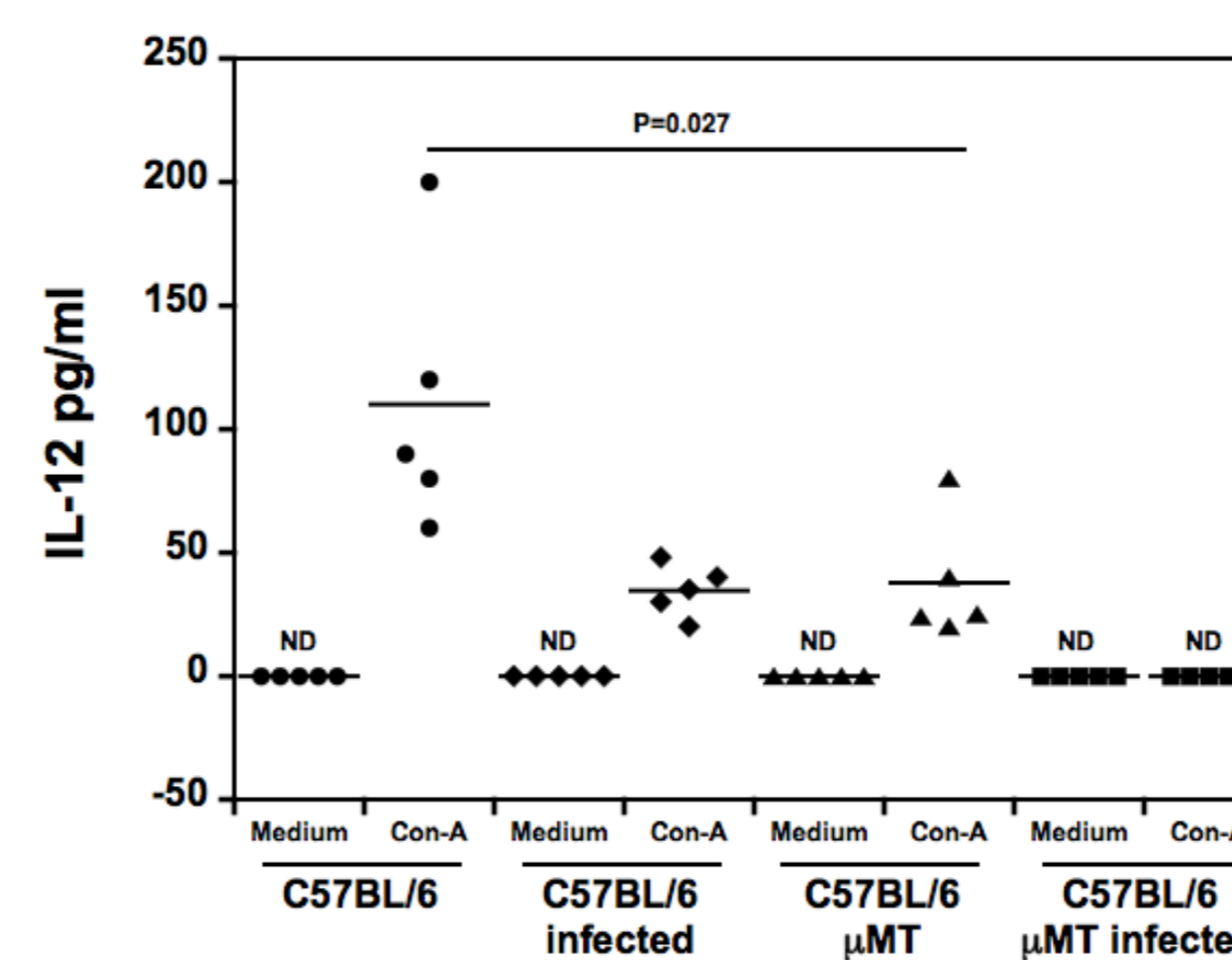


Figure 4A. The production of IL-12 was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.

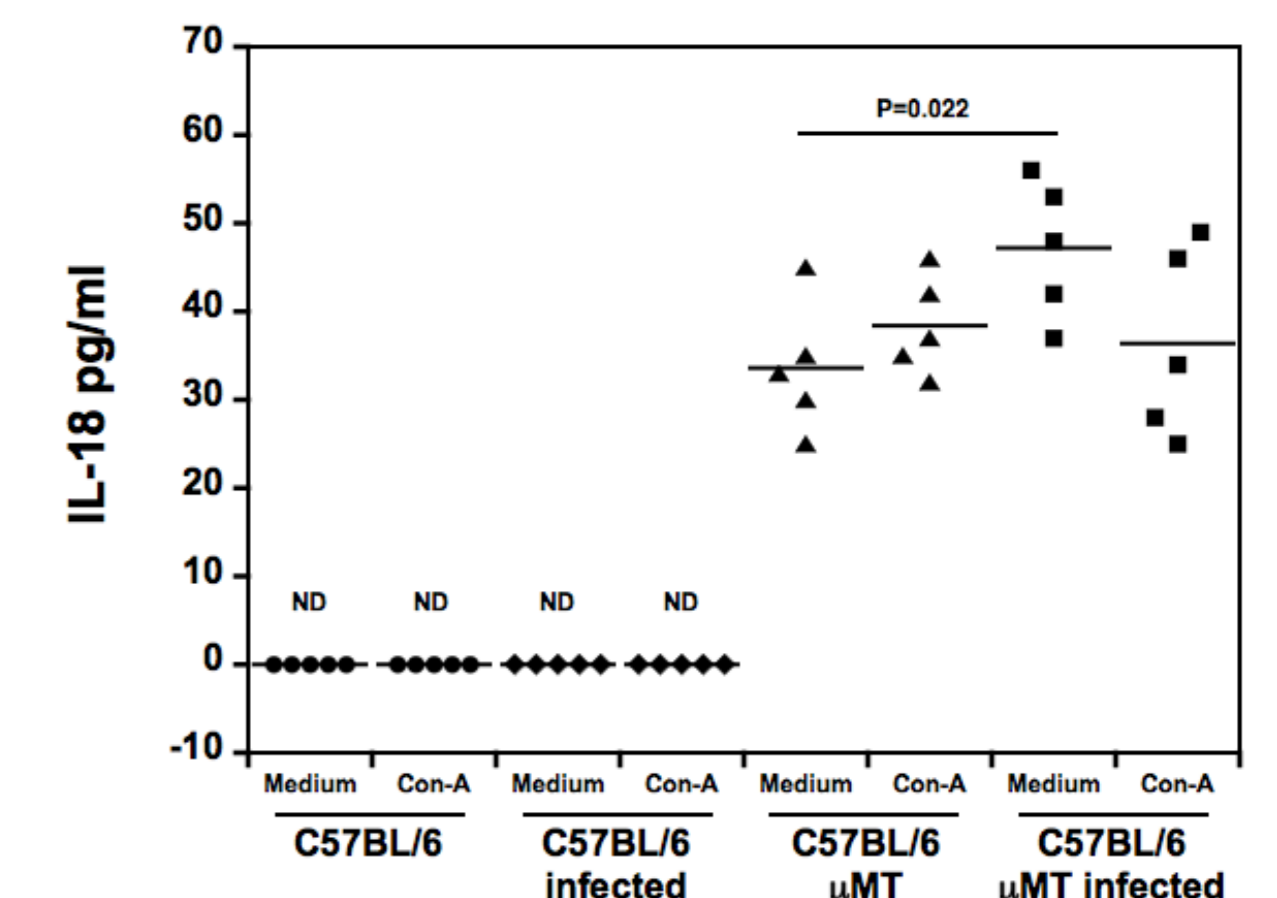


Figure 4B. The production of IL-18 was measured by ELISA assays in supernatants from splenic cells after 48hs in medium alone or in the presence of Con-A. Mice were studied individually, N=5 per group. ANOVA/Bonferroni test was applied.

#### 5- B-cell deficient mice have increased inflammatory infiltrate during the chronic phase of *T. cruzi* infection.

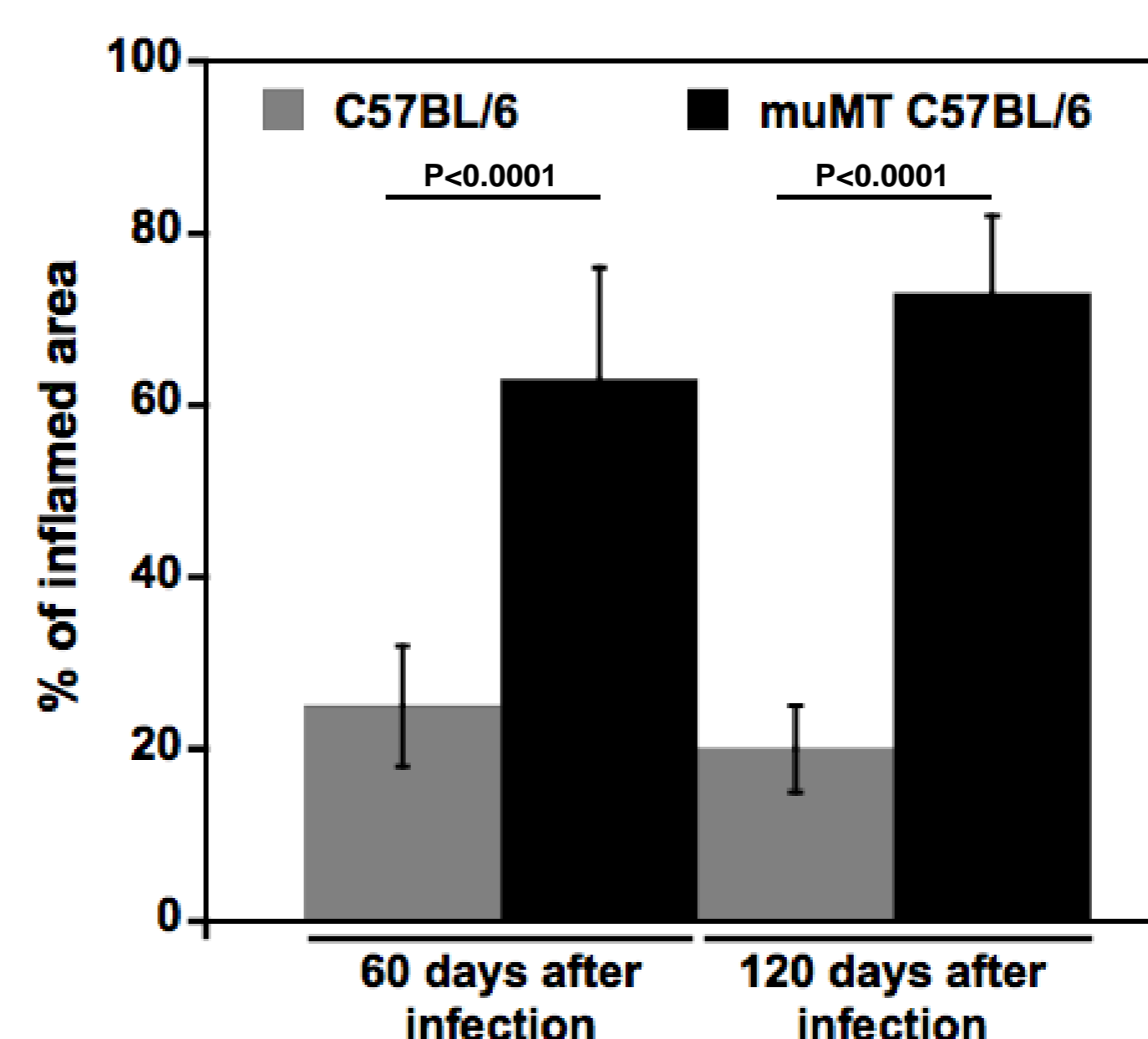


Figure 5. Wild-type C57BL/6 or muMT KO C57BL/6 mice were infected *i.p.* with 50 trypomastigote forms of Tulahuen strain of *T. cruzi*. Quantitative analysis of inflammatory infiltrates in skeletal muscle tissues were evaluated in 10 non-consecutive histological sections obtained from mice at day 120 after infection. Results are shown as mean  $\pm$  SD. Mann-Whitney test was used to compare indicated groups.