Effects of TNF-alpha Inhibition On A Mouse Model Of Controlled *Coccidioides* Infection

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INTRODUCTION

Among the biological disease-modifying antirheumatic drugs utilized for treatment of a variety of immune-mediated inflammatory diseases, the first and most commonly used are TNF-α inhibitors (TNFi) : infliximab (Remicade), adalimumab (Humira), golimumab (Simponi), and etanercept (Enbrel). All TNFi have been reported to be associated with severe *Coccidioides* infections though not all patients treated with these drugs who become infected with *Coccidioides* experience severe disease. The underlying biological mechanisms that could explain the diversity remain unknown, but could include immunogenetic diversity, previous exposure to *Coccidioides* and comorbidities. Development of a mouse model to dissect the key components in these mechanisms is an opportunity for rapid discovery.

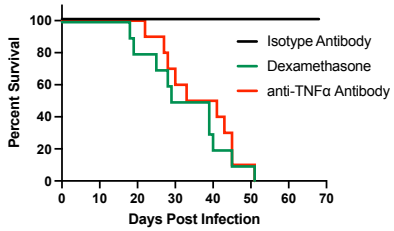
MATERIALS AND METHODS

C57BL/6NJ X DBA/2J (B6D2 F1) mice, which experience long term control of disease when given *C. posadasii* strain 1038, were inoculated intranasally with 50 spores. To test the effect of TNFα suppression on coccidioidal infection, anti-TNF-α monoclonal antibodies or an isotype antibody control were administered intraperitoneally (ip) twice weekly starting 2 days prior to infection and continuing until euthanasia. As a positive control for immunosuppression, mice were administered dexamethasone in drinking water. Mice were monitored for disease progression and euthanized when moribund if prior to end of study

In a second study, mice were administered anti-TNF-α antibody as above starting 2 days before infection and treatment was continued for either 2 weeks post infection or for the duration of the experiment. Mice were sacrificed at 28 days post infection or allowed to progress for up to 70 days post infection. Upon sacrifice lung and spleen fungal burdens were determined by serial dilution.

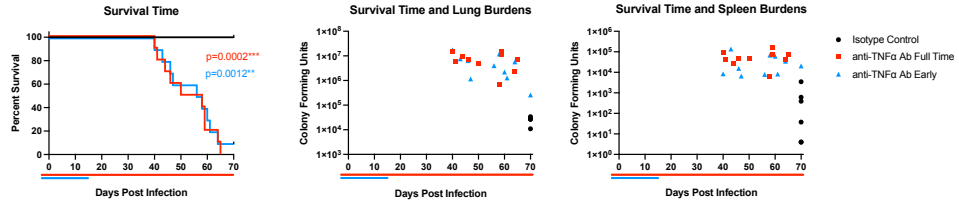
For a final study B6D2 F1 mice were infected with Cp1038 and anti-TNF-α antibody treatment was started 42 days after infection. At 70 days post infection mice were sacrificed and lung and spleen fungal burden was determined by serial dilution.

RESULTS



B6D2 F1 mice are susceptible to Cp1038 when immunosuppressed

B6D2 mice (n=10/group), were given 500μg anti-TNFα antibody q3/q4 (red line), 500μg isotype control q3/q4 (black line), or Dexamethasone in drinking water (green line) starting 2 days before infection. On day 0 mice were infected intranasally with 50 spores of Cp1038 and followed for disease progression.

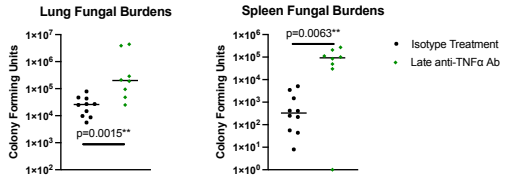


Mice receiving anti-TNFα antibody have more rapid disease progression and increased fungal burdens regardless of stopping antibody treatment.

B6D2 mice (n=8-10/group), were given anti-TNFα antibody (red squares or blue triangles) or isotype control (black circles) starting 2 days before infection. On day 0 mice were infected intranasally with 50 spores of Cp1038 and followed for disease progression. Therapy was stopped on day 14 post infection for a subset of mice (blue triangles). Antibody treatment length is indicated with the solid bars below the graphs. As mice became moribund or 70 days after infection mice were sacrificed and fungal burdens were determined in the lung and spleen by serial dilution. Significance was determined using Mantel-Cox analysis.

Late TNFα antibody treatment inhibits continued host control of Coccidioides

On day 0 B6D2 mice (n=10/group), were infected intranasally with 50 spores of Cp1038. On day 42 post infection mice were then treated with isotype control (black circles) or anti-TNFα antibody (green diamonds) on day 70 post infection mice were sacrificed and fungal burdens were determined in the lung and spleen by serial dilution. Significance was determined using a Student's t-Test on log transformed data.



CONCLUSIONS

The B6D2 F1 mouse model coupled with Cp1038 allows for examination of the effects on immunosuppression on both the initial host response as well as host factors critical for ongoing control of *Coccidioides*. Mice receiving immunosuppression, whether broad (dexamethasone) or targeted (anti-TNFα) rapidly succumbed to infection. Strikingly, there was no difference in the time to death of the treatment groups, indicating that TNFα has a critical role in the host response to *Coccidioides*.

To further examine the effect of timing of anti-TNFα therapy mice were treated with anti-TNFα antibody for either just the first two week of the infection or for the duration of the experiment. Surprisingly, even though mice were only treated the first two weeks of infection they succumbed to infection and had similar fungal burdens compared to mice receiving treatment for the entire experiment. Taken together this indicates a role for TNFα early in infection that is not remedied by stopping treatment after infection.

Finally we sought to determine if there was a role for TNFα in the ongoing control of Cp1038 by B6D2 F1 mice. Mice were infected with Cp1038 and starting on day 42 post infection were treated with anti-TNFα antibody or control antibody. 70 days after infection mice were sacrificed and fungal burdens were determined in the lung and spleen. Mice receiving antibody treatment had significantly higher fungal burdens in the lung and spleens as compared to control treated mice. While we previously showed a role for TNFα early in the host response this experiment highlights an additional role late in host control. The particular host changes that are influenced by this anti-TNFα intervention are the subject of ongoing studies.

ACKNOWLEDGEMENTS

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