Role of Natural Killer Cells in Adaptive Immunity to *Toxoplasma gondii*

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Abstract

Natural killer (NK) cells are necessary members of the immune system to fight invading viral and cellular pathogens. Recent research has demonstrated a dual role of NK cells in both innate and acquired immunity. Our goal was to determine if NK cells play a significant role in adaptive immunity to Toxoplasma *gondii*. We demonstrated that NK cells are necessary for survival and control of the parasite following a secondary exposure to T. *gondii.*

Introduction

Toxoplasma *gondii* is an obligate intracellular parasite that infects an estimated 22.5% of the American population. While immune competent individuals are largely asymptomatic, reactivation of chronic toxoplasmosis is the third leading cause of AIDS related death. In addition, chronic T. *gondii* is also associated with the development of Schizophrenia, depression and dementia. Natural killer (NK) cells are widely known to provide a significant innate immune response to invading pathogens like T. *gondii.* However, NK cells have recently been shown to play a large role in adaptive immunity (Cooper et al, 2009).

The previous demonstration That NK cells persist to provide an adaptive response in other models such as; Hapten induced DTH, murine cytomegalovirus (MCMV) and Plasmodium (Malaria), led us to hypothesize that a similar response could occur in the T. *gondii* model (Paust & Von Adrian, 2011). Toxoplasma is an ideal model to test adaptive immune function because the immunization strain not only produces a robust immune response, but is also 95% cleared after 5 weeks. This creates an optimal situation for studying adaptive immunity due to the lack of persistent parasite activity from the initial infection. To test this hypothesis we exposed mice to Toxoplasma, allowed them to recover, then depleted NK cells and exposed them to another infection. We looked for differences in survival based on the presence or absence of NK cells.

Figure 1. % Survival After Type I *Toxoplasma gondii* Rechallenge in Type II Infected Mice

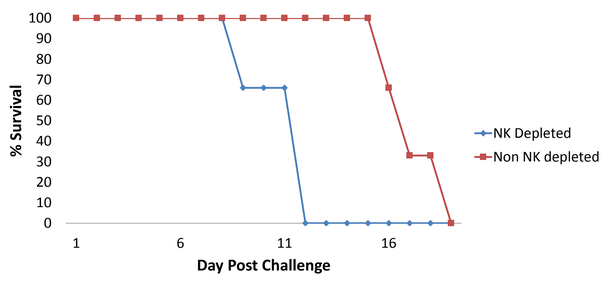

Figure 1. Absence of NK cells shortened survival time in a type I rechallenge after type II infection of T. gondii. Six C57BL/6 mice were infected with 10 cysts of type II T. gondii i.g. All mice were infected will 1000 tachyzoites 6.5 weeks post infection i.p. Three mice were also depleted by injecting 50µL of α-Asialo GM-1 i.p. every third day for 18 days (blue line), while three mice were left untreated (red line). This experiment was conducted once with n=3 for each group

Figure 2. % Survival After Type II *Toxoplasma gondii* Rechallenge in Type II Infected Mice.

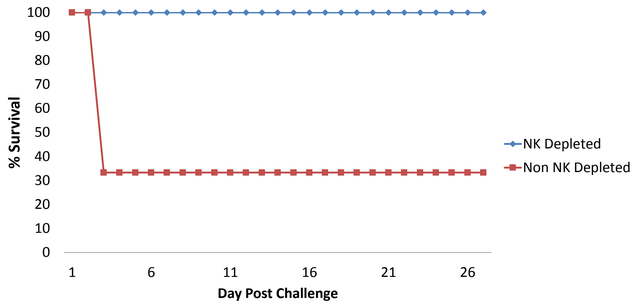

Figure 2. Absence of NK cells in type II *T. gondii* rechallenge showed no impact on survival. Six C57BL/6 mice were infected with 10 cysts of the ME49 strain. After five weeks of infection the NK cells from three mice were depleted by injecting 50µL α-Asialo GM-1 i.p. every third day for 21 days (blue line) while three mice were left untreated (red line). Additionally all mice were rechallenged with 100 cysts of the ME49. Survival is currently being monitored for five weeks post rechallenge. This experiment was conducted once with n=3 for each group.

Figure 3. % Survival of α-NK1.1 Treated *Toxoplasma gondii* Immunized Mice After Type I or II Rechallenge.

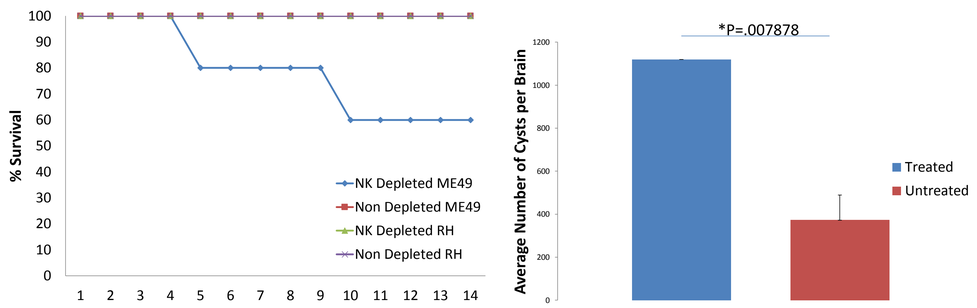

Figure 3. NK cells are necessary for survival after immunization and Type II T. gondii rechallenge. Twenty C57BL/6 mice were immunized with the attenuated CPS strain of T. gondii. Five weeks after immunization ten mice were infected with 5000 tachyzoites i.p. of the RH strain, depleted (green line) untreated (purple line), and ten were infected with 20 cysts i.g. of the ME49 strain, depleted (blue line) untreated (red line). Additionally the NK cells from five mice in each group were depleted by injecting 200µg of α-NK1.1 i.p. every other day for 21 days. Survival was then monitored for 35 days post infection. Additionally, at 35 days the number of brain cysts were counted in 3 ME49 infected mice from each group and the rest were allowed to run the course of their infection. This experiment was conducted once with n=5 for each group and the statistical significance of cyst counts was calculated with a students T-test with a P-value less than 0.05.

Figure 4. % Survival of α-Asialo GM1 Treated *Toxoplasma gondii* Immunized Mice After Type I or II Rechallenge.

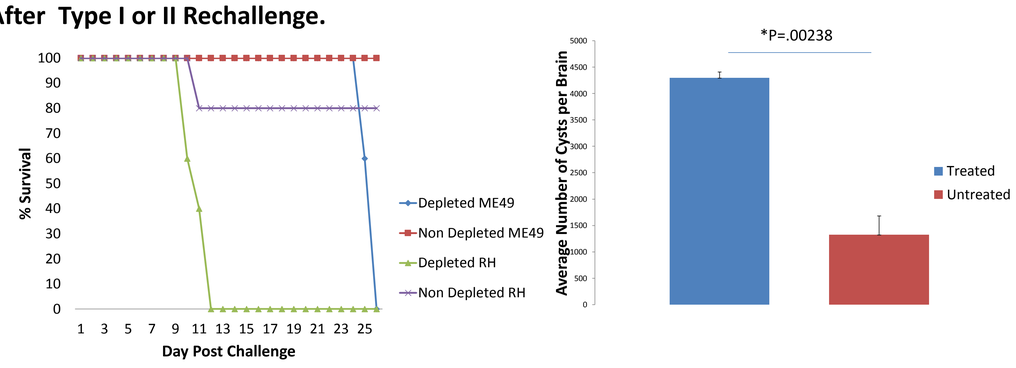

Figure 4. NK cells are necessary for survival during T. gondii infection after immunization. Twenty C57BL/6 mice were immunized with the attenuated CPS strain of T. gondii. Five weeks post immunization ten mice were infected with 1000 tachyzoites i.p. of the RH strain and ten were infected with 100 cysts i.g.of the ME49 strain. Additionally the NK cells from five mice in each group were depleted using 50µL α-Asialo GM-1 i.p. every third day for 21 days. Survival was then monitored for 35 days post rechallenge. For the ME49 infected mice brain cyst counts were obtained after death or after 35 days post rechallenge for surviving mice. This experiment was conducted once with n=5 for each group and the statistical significance of cyst counts was calculated with a students T-test with a P-value less than 0.05.

Summary

The experiments starting with a type I infection are a model for a real world human infection however they did not effectively show that NK cells are necessary for an effective immune response upon a second challenge. This is most likely due to the fact that parasites from the initial infection were just beginning to reactivate when the mice were challenged for a second time. Due to these results we switched to the CPS immunization strain that has been proven to illicit a very strong immune response to T. gondii (Jordan et al,2009). After switching the initial exposure to the immunization strain we found that NK cell deficient mice did not control the parasite as well in ME49 infection. However there was not significant evidence to suggest that NK cells are necessary in survival particularly with the RH strain. So, we changed to the more potent α-Asialo GM-1 antibody to deplete NK cells and increased the dose of ME49. After this optimization we observed 100% death of NK cell deficient mice after rechallenge regardless of strain. In addition there was statistically significant increase in parasite burden for NK cell deficient mice. These findings indicate that NK cells are likely playing a significant role in mounting an adaptive immune response to T. *gondii.* However it is not yet clear if the improved survival is due to persisting NK cells or newly activated naïve NK cells.

Future Directions

- Conduct adoptive transfer experiments to confirm that NK cells acquire long term survival.
- Conduct adoptive transfer experiments to test whether NK cells from *T. gondii* infected mice are more active in response to secondary infection in naïve animals

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