Spring 5-10-2017

Understanding the Role of Natural Killer Cells on the Quality of CD4 and CD8 Response During Infection with Toxoplasma gondii

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Understanding the Role of Natural Killer Cells on the Quality of CD4 and CD8 Response During Infection with *Toxoplasma gondii*

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Abstract

30% of the world's population is infected by the obligate intracellular parasite *Toxoplasma gondii* including, more than 60 million people in the USA alone (CDC 2015). Toxoplasma poses a critical threat to individuals with a weakened immune system such as HIV/AIDS patients (Mayo Clinic 2014). In addition, severe birth defects, blindness, and abortion can occur in the fetus when the parasite is transmitted to healthy mothers who are pregnant (Sibley 2012). Thus, understanding how long term immunity develops against this parasite is important for better therapy design.

Natural Killer cells (NK cells) are known to be important for early protection against Toxoplasma. Recently, in persistent viral infection NK cells have been shown to inhibit priming of CD4 and CD8 T cells during acute infection. We sought to understand whether this was happening during acute *T. gondii* infection. We tested whether NK cells impact the development of the protective T cell response. We observe the presence of NK cells helps boost early CD4 and CD8 T cell responses. Depletion of NK cells with a low or high dose of NK depleting antibody significantly reduced the T cell's ability to produce the cytokine IFNγ. Reduced T cell responses were also observed in NKT cell deficient animals. Our results indicate both NK and NKT cells are required for priming optimal T cell responses against the parasite and demonstrate the role of NK cells in parasite infection are different than their role in viral infection.

Introduction

Vaccines are vital in our public health endeavors. The development of vaccines has led to a great reduction in many diseases along with the eradication of some. Vaccines utilize memory functions of immune cells (B and T cell), to expose and prepare the body against infection with a pathogen (Rydyznski, Carolyn, et al 2015). Unfortunately, a great number of pathogens still exist that we have not been able to develop vaccines against due to their unique methods of evading the immune system. It is crucial for us to expand our knowledge of the immune system and innovate ways to target many diseases that we have been unable to before. Based on research in viral models, Natural Killer (NK) cells have shown intriguing behaviors affecting the role of B and T cells and how they respond to pathogens (Rydyznski, Carolyn, et al 2015). It is highly important to explore this avenue in the search for novel solutions to many diseases that affect us.

Toxoplasma gondii is an obligate intracellular parasite and found in 30% of humans around the world (Sibley et al. 2012). Toxoplasma can be transmitted to humans via three routes: foodborne transmission, zoonotic transmission (animal to human), and through congenital transmission (mother to child) (Yarovinsky 2014). To date, there are no effective therapies or vaccines available that can prevent or clear Toxoplasma infection and exposure of people to *Toxoplasma gondii* can be problematic. Toxoplasma infection is lifelong and many health problems arise due to reactivation of chronic infection. Generally, the immune system can control the parasite and the infected individual develops flu-like symptoms if any at all. However, this parasite poses a significant risk for pregnant women and people with a compromised immune system such as HIV/AIDS patients and those undergoing chemotherapy (Mayo Clinic 2014).

The whole immune response is important for defense against *T. gondii* but, the relationship between NK cells, T cells, and NKT cells was further examined. NK cells are lymphocytes that are a part of the innate immune system, they play a crucial role in the immune response against infectious diseases (Caligiuri 2008). With exposure to a pathogen, NK cells release chemicals to destroy the pathogen (known as cytotoxicity) and release chemicals to warn other host defenses, this includes pro-

inflammatory cytokines such as Interferon-gamma (INFγ) and TNF-α (Vivier et al. 2016). Not only does NK cell cytotoxicity affect pathogens, it has shown to have effects on other immune cells in some viral models. Some properties of NK cells such as the production of INFγ has shown to improve the generation of T cell memory however, natural killer cells can also hinder adaptive immunity through immunosuppressive cytokines in persistent viral infections (Rydyznski, Carolyn, et al). Though the original perspective considered NK cells as only a part of the innate immune system, this is changing because NK cells have shown the development of some memory-like traits and as such are involved in not only short term immunity, but long term immunity as well (Sun et al. 2014).

T cells are part of the adaptive immune system; they require activation but lead to a longer-term response. To become activated, antigen must be presented to the T cell receptor via an antigen presenting cell. T cells are generally divided into two distinct populations, CD4 or CD8 T cells. CD4 or helper T cells secrete cytokines (such as INFγ) and enhance the response of other immune system cells. CD8 or cytotoxic T cells tend to perform a killing function when non-self is detected (University of Washington). NKT cells, on the other hand, pose more of an enigma. First discovered in 1995, NKT cells share characteristics with both NK and T cells (Godfrey, DI, et al., 2004). Originally, NKT cells were defined as lymphocytes with both NK1.1 marker and T cell receptor, however, as more categories of NKT cells are discovered, they are seen as a heterogenous population and precise characterization remains to be very difficult (Gapin and Kronenberg, 2002).

Studies conducted in viral models have shown that highly activated NK cells can reduce T cell response and NK cell depletions could serve as a therapeutic approach which would allow the reactivation of T cell responses and control of the virus (Waggoner et al., 2014). No such response has yet been characterized in relationship to *Toxoplasma gondii* during acute infection. We tried to characterize the interaction between T cell and NK cell, in the presence and absence of NKT cells, by performing low and high dose NK cell depletions and studying the effects on T cell activity. Based on viral research, we hypothesized that NK cell depletions would lead to an improvement in T cell response.

Methods

Infection and Depletion

Two different groups of mice were obtained, Wildtype (CD45.2) and NKT Cell deficient mice (CD1 knockout). Both groups of mice were infected orally with 10cysts of ME49 strain of *Toxoplasma gondii*. Within each of the two groups, 5 mice were infected and received no treatment, 5 mice were infected and received a low dose NK cell depletion, and 5 mice were infected and received high dose NK cell depletion. The low dose depletion entailed 50µg of anti-NK1.1 antibody administered just once, intraperitoneally, a day before the infection. High dose depletion involved intraperitoneal injections containing 200µg of antibody on the day before the infection, the day of the infection, and every other day until the mice were harvested. 5 naïve mice that had no depletions or infections were used as the negative control.

Harvest and Flow Cytometry

Seven days post infection, the mice were euthanized and their spleens were harvested. Splenocytes were obtained by crushing the spleens through a 70µm tissue strainer and then lysing the red blood cells. Single cell suspensions of the splenocytes were made and plated at $1x10^6$ cells per well. The cells

were stimulated overnight with Toxoplasma lysate antigen (TLA). Next, the cells were surface and intracellularly stained for T cells markers (CD4, CD8, and INFγ) and analyzed by flow cytometry.

Results

To first try to examine the relationship between NK Cell and T Cells, the number of T Cells in the presence or absence of NK and NKT Cells were quantified (figure 1). Naïve mice that were not infected or treated were used as the negative controls. The results showed that NK or NKT Cell absence had no effect on the amount of T Cells present. Next, the activity of T Cells was studied through the production of INFγ (figure 2).

The results displayed that the low dose NK cell depletion significantly reduced CD4 T cell response whereas, a high dose depletion caused no change. This same pattern was observed in the CD4 T cells in the CD1 knock out mice where the low dose depletion resulted in lower T celll activity but the high dose depletion had no effects. Looking at the effects of the NK Cell depletions on the CD8 T Cells, it can be seen that both (low and high dose) depletions resulted in some decreased activity. For CD8 T cells, a low dose depletion of NK Cells lead to a much greater decrease in activity whereas, the loss in activity with a high dose depletion is much less serve. Again, this pattern of decreased CD8 T cell activity was mimicked in the CD1 knock out groups. Holistically comparing the groups of wildtype and CD1 knock out mice, it can be observed that every treatment group in the CD1 knock out mice has lower activity to its wildtype counterpart.

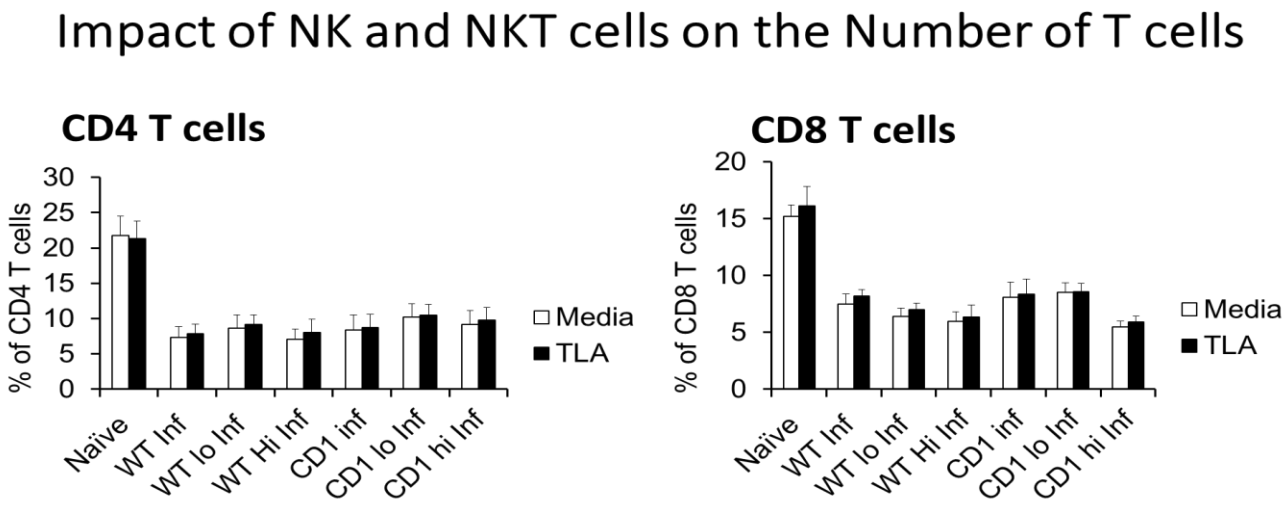

Figure 1. NK cells and NKT cells do not impact the number of CD4 or CD8 T cells during acute *T. gondii* infection. The frequency of CD4 T cells present in the spleen after infection with 10 cysts of the Type II parasite strain ME49. The frequency of CD8 T cells present in the spleen after infection with 10 cysts of the Type II parasite strain ME49. N=5

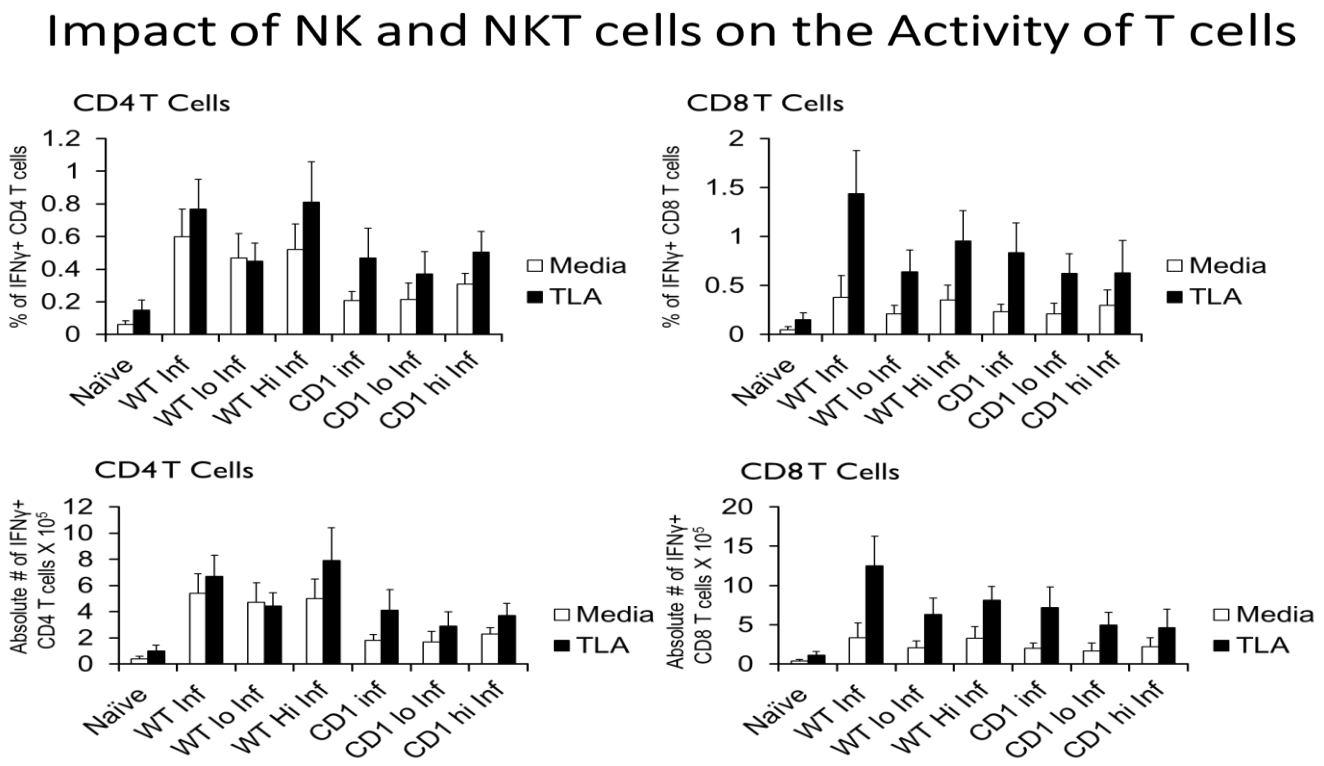

Figure 2. The absence of NK cells and NKT cells reduce the frequency and absolute numbers of activated CD4 and CD8 T cells. (First Column) The frequency and absolute # of IFNγ+ CD4 T cells in spleens 7 days post infection with 10 cysts of ME49. (Second Column) The frequency and absolute number of IFNγ+ CD8 T cells in spleens 7 days post infection with ME49. N=5 per group.

Conclusion

Based on viral models highlighting an interesting interaction between NK cells and T cells, we sought to characterize the interaction occurring between NK cell and T cell in an acute *Toxoplasma gondii* infection. Due to the vast similarities NKT cells have with both T cells and NK cells, NKT cells were also considered in this study. The group of CD1 knock out mice was selected to control for the effects of NKT cells. Normally, when NK cell depletions are performed, NKT cells are also affected. To solely study the interaction between NK cells and T cells, CD1 knock out mice were used.

The first thing shown was that NK and NKT cells do not affect the number of T cells present. Preceding the study, it was hypothesized that depleting the NK cells would lead to an improvement in T cell response, however, the opposite was observed. A low dose depletion of NK cells lead to significantly reduced CD4 and CD8 T cell activity. A high dose depletion of NK Cells resulted in either no effect (CD4 T cells) or a slight reduction (CD8 T cells) in activity. This shows that NK cells are required for a proper T cell response. The the reduced relationship between a high dose NK cell depletion and T cell activity is incredibly interesting and poses an avenue for further research. Another key trend observed in the results is that all the CD1 knockout groups have reduced T cell activity when compared to the same group in wildtype mice. This indicates that NKT cells are also needed for proper T cell response.

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