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Role of IL-17 in CD8+ T Cell Mediated Clearance of West Nile Virus

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by

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Abstract

West Nile virus (WNV), a positive sense single stranded RNA flavivirus, is prevalent in many countries worldwide and is a growing threat in the United States. Some patients infected with WNV develop severe neuroinvasive disease including meningitis or encephalitis which may lead to death. The recently characterized cytokine interleukin-17 (IL-17) has been implicated in several autoimmune diseases, neuroinflammatory conditions, and immune responses to various microbial infections. Previous research has indicated that a known contributor to IL-17 production, interleukin-23, is produced in response to WNV infection, but the role of IL-17 during WNV infection has not been studied before. This study found that WNV infected IL-17 deficient (IL-17-/-) mice developed higher viral loads in the blood and brain compared to wild-type (WT) control mice and were susceptible to severe WNV infection (IL-17-/- 20% survival; WT 60% survival). Interestingly, CD8+ T cells isolated from IL-17-/- mice infected with WNV showed reduced cytotoxicity compared to WT CD8+ T cells. These results suggest that IL-17 plays a protective role during WNV infection by contributing to the cytotoxicity of CD8+ T cells. CD8+ T cells have previously been shown to play a protective role during WNV infection, but little is known about the activation CD8+ T cells or their recruitment to WNV infected cells. Further studies are warranted to elucidate the mechanism by which IL-17 signaling regulates CD8+T cell responses during WNV infection.

Key words: West Nile virus, CD8+ T cells, Interleukin-17

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List of Abbreviations

BSL3	Biosafety Level 3
CD	Cluster of Differentiation
CNS	Central Nervous System
cDNA	Complementary DNA
DC	Dendritic Cell
Fas-FasL	Fas-Fas Ligand
IL-17-/-	IL-17 deficient
IL-17	Interleukin-17
IL-23	Interleukin-23
IFN	Interferon
i.p.	Intraperitoneal
PFU	Plaque Forming Units
p.i.	Postinfection
qPCR	Quantitative Polymerase Chain Reaction
TLR7	Toll-like Receptor 7
WNV	West Nile Virus
WT	Wild Type

Chapter 1: The Problem

West Nile virus (WNV) is an emerging viral infection worldwide and a growing threat in North America. WNV is primarily maintained through avian-mosquito transmission cycles and transmitted to human hosts by the bite of an infected mosquito. Once inside the human body, WNV can cause a number of symptoms that may range from minor febrile illness to fatal encephalitis. WNV poses a more serious threat to older and immunocompromised patients in which the disease often presents as fatal encephalitis. Currently, there is no specific antiviral drug or vaccine available to treat WNV infections in humans. In addition, the pathogenesis of WNV infection and the role of the host immune system are still not adequately investigated. Understanding of the host's defense mechanisms to fight WNV infection can lead to the development of novel therapeutics and vaccines.

The immune system responds to WNV infection in a number of ways. Among various innate and adaptive immune effector mechanisms, CD8+ T cells play a prominent role in combating viral infections. Previous studies have shown that CD8+ T cells play an important role in the clearance of WNV and protection from fatal WNV infection. These cytotoxic CD8+ T cells act via targeted killing of WNV infected cells using various mechanisms including Fas-Fas Ligand (Fas-FasL) interaction and the release of preformed cytotoxic granules. However, the exact mechanism of CD8+ T cell activation, recruitment to infection sites, and virus clearance is not completely understood and requires further study.

Several components of the immune system, including cytokines and chemokines, are produced during infection and determine fate of CD8+ T cells. This

includes the activation, migration and cytotoxic functions of these cells. It has been demonstrated that a newly characterized cytokine, interleukin-17 (IL-17), plays an important role in most immune responses by promoting inflammation and recruiting neutrophils. In addition, studies have shown that cells involved in production of IL-17 contribute to clearance of other viruses and intracellular pathogens.

A population of CD8+ T cell (called Tc17), which produce IL-17, has been shown to provide protection during influenza virus infection. Tc17 cells have been found to secrete IL-17 during vaccinia virus and hepatitis C virus infection, as well. In addition, IL-17 has been found to promote CD8+ T cell activation during infection with the intracellular bacteria *Listeria monocytogenes*. However, the role played by IL-17 has not previously been studied in relation to WNV infection. Preliminary studies in Dr. Fengwei Bai's laboratory have shown that IL-17 deficient (IL-17-/-) mice are more susceptible to WNV infection compared to wild-type mice. It follows that IL-17 could play a role during the CD8+ T cell mediated immune response to WNV. Therefore, I hypothesized that IL-17 plays a role in the CD8+ T cell mediated immune response to WNV. IL-17 is produced by several immune cells including γδ T cells and Th17 cells. IL-17 is shown to be produced during WNV infection, and γδ T cells, a major source of IL-17 during infection, play a protective role against WNV infection. Whether IL-17 producing CD8+ T cells function in clearing WNV infections or IL-17 produced by other cell types aid in the activation and migration of CD8+ T cell has not been described during WNV infection.

In order to elucidate the function of IL-17 in controlling CD8+ T cell function, a mouse model of WNV infection (wild-type (WT) and IL-17 -/- mice) was

used. To this end, the research planned to fill in some of the unknown areas in CD8+ T cell mediated clearance of WNV including the role of IL-17 in the activation and cytotoxic function of the CD8+ T cells. This research also aimed to identify a previously undescribed function of IL-17 in protecting against severe WNV infection through activation of CD8+ T cells. This novel IL-17-CD8+ T cell axis may be applicable to further understanding of WNV pathogenesis and to development of novel therapeutics.

Chapter 2: Literature Review

2.1: Background

West Nile Virus (WNV) is a positive sense, single-stranded RNA (+ssRNA) flavivirus. It was first discovered in Uganda in 1937, and has since been reported in many countries around the world. The first instances of WNV infection in the United States were found in 1999, and the virus quickly spread across the country (Brault, 2009). The North American strain is highly infectious and causes a high viremia in birds. This strain also seems to be more virulent than the strain originally discovered in Africa (Brault, 2009). WNV is maintained in an avian-mosquito transmission cycle and is transmitted from birds to humans via infected mosquito vectors of the *Culex* genus (Wertheimer, 2012; Brault, 2009). The transmission cycle of WNV is shown (Fig. 1).

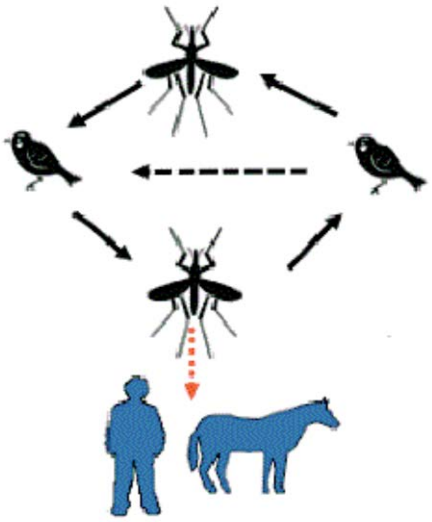

Figure 2.1. The transmission cycle of WNV. Primary transmission cycle shown in black, incidental hosts shown in blue. *(Adapted from Brault (2009), p. 47)*

In humans, most infections with WNV are asymptomatic and do not require

treatment (Sitati & Diamond, 2006). However, symptoms vary widely among patients, and in some cases, infection can cause meningitis, encephalitis, paralysis, or even death (Sejvar et al., 2003). In young patients, infection generally presents as West Nile fever (Brault, 2009). WNV can also invade the central nervous system (CNS) in less than 1% of patients, usually elderly or immunocompromised individuals, and lead to severe neurologic symptoms (Sejvar et al., 2003). Due to the possibility of severe symptoms, more knowledge of the mechanisms of WNV infection and the immune response is needed in order to provide optimal healthcare to infected patients.

2.2: WNV Pathogenesis and Immunity

After inoculation through the bite of an infected mosquito, WNV is thought to infect the Langerhans cells in the skin (a type of dendritic cell) then move to the lymph nodes. After one week, the virus can be detected in the CNS in some infected patients (Samuel & Diamond, 2006). WNV causes apoptosis of host cells and by this mechanism can cause death of the host, especially when cells of the CNS are infected (Wang et al., 2006). In a mouse model of WNV infection, Wang et al. (2003) found that virus can be isolated from lymphoid tissues 4-5 days after infection and can be isolated from the brain 7 days after infection, when a dose is administered at a similar size to that of a mosquito vector bite. Though it is mainly passed through arthropod vectors, it is possible to pass the virus by infected organ transplant or whole blood donation without proper screening for WNV (Rhee et al., 2011). No vaccine has been approved for human use, though there are equine vaccines available. Currently the most effective means of controlling WNV in humans is through control of the mosquito population (Wertheimer, 2012). Therefore, development of effective clinical strategies is critical to combating WNV infection in

humans.

Inside the host's body, WNV elicits a response from both the innate and adaptive immune systems. In the innate immune response, viral sensors in many cell types detect viral ribonucleic acid resulting in activation of interferon (IFN) and other transcription factors. IFNs provide direct antiviral responses and further stimulate dendritic cell (DC) maturation. DCs can then activate B and T lymphocytes through antigen presentation (Samuel & Diamond, 2006). Several innate immune cells including macrophages, γδ T cells, and natural killer cells directly kill WNV infected cells (Samuel & Diamond, 2006). While these innate immune mechanisms respond during the early period, the adaptive immune response develops later to further control WNV infection.

In the adaptive immune response, B lymphocytes produce antibodies IgM and IgG, which control viremia and inhibit further dissemination to CNS. These antibodies are thought to neutralize the virus, blocking its entry into host cells (Samuel & Diamond, 2006). Similarly, CD4+ or CD8+ T lymphocytes also provide immunity to WNV infection and play an important role in host recovery. CD4+ T cells act by priming of CD8+ T cells, secretion of cytokines, activation of B cells, and direct cytotoxicity. CD8+ T cells respond to WNV infection by secretion of inflammatory cytokines and direct killing of infected cells. All of these lymphocytes also contribute to memory responses in future WNV infections (Samuel & Diamond, 2006). An overview of the adaptive immune response to virus infection is given (Fig. 2).

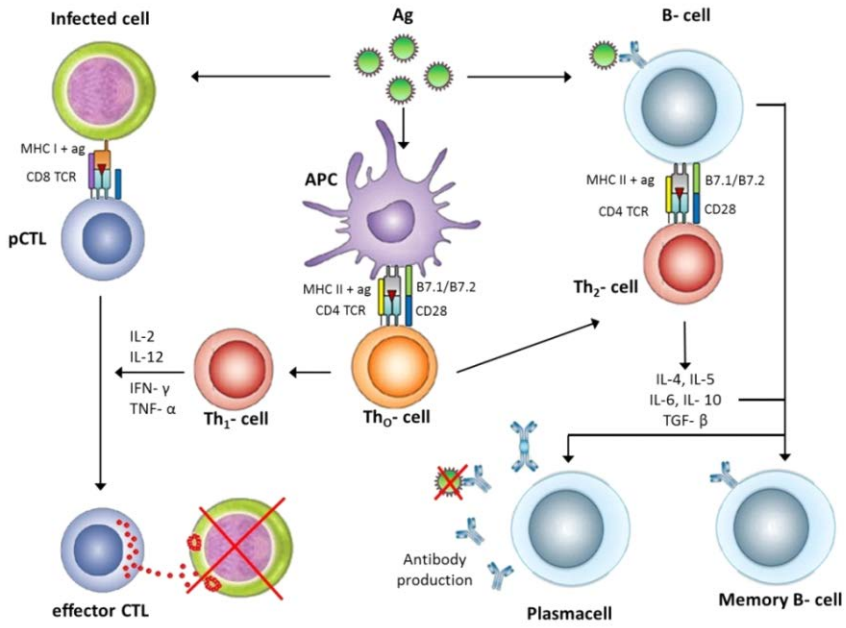

Figure 2.2. Adaptive immune response to virus infection. *(Adapted from De Haes (2012), p. 31)*

2.3 Role of CD8+ T Cells during WNV Infection

CD8+ T cells, also referred to as cytotoxic T lymphocytes, play a critical role in control of WNV infection. These are comprised of both effector cells that directly fight the current virus infection and memory cells that aid the body in fighting future infections (Cox et al., 2013). According to Shrestha and Diamond (2004) mice that lack normal levels of CD8+ T cells are more likely to die from WNV infection, indicating that these cells are important to WNV clearance. CD8+ T cells combat WNV infection in several ways including release of cytotoxic granules, Fas-FasL interaction, production of antiviral cytokines and tumor necrosis factor alpha (Shrestha & Diamond, 2007), but evidence has shown that they may also utilize other killing methods.

In a study of the progression and clearance of WNV, Wang et al. (2003) found that CD8+ T cells were the dominant type of lymphocyte that homed to the brain after WNV infection. From their findings, the group concluded that CD8+ T cells have an

important role in WNV infection. Shrestha and Diamond (2004) also confirmed that CD8+ T cells play a crucial role in clearance of WNV and use a perforin dependent mechanism to clear WNV from host tissues (Shrestha et al., 2006). However, CD8+ T cells do not use perforin alone to stop the replication of WNV in host cells. Fas-FasL interaction was found to be used by CD8+ T cells in addition to the perforin mechanism (Shrestha & Diamond, 2007). The Fas-FasL mechanism was found to be subordinate to the perforin mechanism and more tissue specific (Shrestha & Diamond, 2007).

These findings confirm that CD8+ T cells play a vital role in the host's response to WNV. Both the perforin and Fas-FasL mechanisms do not show strong phenotypes in WT animals compared to those in CD8+ T cell deficient animals, suggesting that other mechanisms also play a role in CD8+ T cell mediated clearance of WNV infection. Although the role of CD8+ T cell during WNV infection is mostly understood, little is known about the mechanisms of CD8+ T cell activation and recruitment to infection sites. Further study is needed to fully elucidate the method of CD8+ T cell activation and recruitment during WNV infection.

2.4: Role of IL-17 in CD8+ T cell Function

The activation and differentiation of antiviral CD8+ T cells is controlled by a number of intracellular signaling molecules called cytokines (Cox et al., 2013). It has been found in the Bai laboratory that IL-17-/- mice are more susceptible to WNV infection than WT mice (Unpublished data). IL-17 is produced by a number of immune cells including Tc17, a subset of CD8+ T cells, and Th17 cells, a subset of CD4+ T cells (Hamada et al., 2009). Other cells that produce IL-17 include γδ T cells, natural killer cells, natural killer T cells, neutrophils, and eosinophils (Korn et al., 2009). These cells

span over both innate and adaptive immunity, making IL-17 active in both systems (Korn et al., 2009).

Cells that produce IL-17 are known to act by promoting inflammation, promoting hematopoiesis, and recruiting neutrophils (Hamada et al., 2009). However, some evidence has shown that IL-17 also plays an important role in the adaptive immune system through the homing of activated CD4+ Th1 cells and CD8+ T cells to infection sites. In addition, Tc17 cells that produce IL-17 have been found to aid in clearance of vaccinia virus in a mouse model, as well as use an interferon gamma dependent mechanism to clear influenza virus (Yeh et al., 2010; Hamada et al., 2009).

A similar effect was found in hepatitis C infection, another member of the flaviviridae family, along with WNV, where Tc17 cells home to the liver and secrete IL-17 while playing a role in hepatitis C virus clearance (Billerbeck et al., 2010). However, it has been shown that Tc17 cells do not produce perforin and use no other means of direct cytotoxicity (Hamada et al., 2009). These findings led to the conclusion that the Tc17 cell functions in the recruitment of other host cells, including CD8+ T cells, instead of acting through direct cytotoxicity (Hamada et al., 2009). Even so, the influence of IL-17 on CD8+ T cells during WNV infection has not been studied. It is possible that IL-17 plays a role in CD8+ T cell mediated immunity during WNV infection.

Besides this, IL-17 has also been found to contribute to the activation of CD8+ T cells. IL-17 is known to be produced by γδ T cells during innate immune response to WNV infection, but little is known about its role in the activation of CD8+ T cells. During *L. monocytogenes* infection, a subset of γδ T cells which produce IL-17 contribute to the proliferation of CD8+ T cells through enhanced cross presentation of

antigens by DCs (Xu et al., 2010). CD8+ T cells are able to respond to the antigens presented on the DCs and become activated to carry out cytotoxic effects. Xu et al. (2010) also suggested that Th17 cells, which are one of the cellular sources of IL-17, aid in proliferation of CD8+ T cells, but the method of which is not fully understood and requires further study. This indicates that the IL-17 produced by other cell types may play a role in proliferation and activation of CD8+ T cells during WNV infection. Further study is required to confirm this hypothesis.

2.5: Hypothesis

CD8+ T cells are known to be important in the body's defense mechanisms against WNV. Further study is required to fully understand the activation and differentiation of these cells. IL-17 has been shown to be effective in CD8+ T cell mediated clearance of other viruses and intracellular pathogens and has been shown to affect the activation of CD8+ T cells. Therefore, it follows that IL-17 may influence the function of CD8+ T cells during WNV infection, as well. Based on these findings, I predicted that IL-17 could play an important role in CD8+ T cell mediated clearance of WNV and tested this hypothesis by comparing the cytotoxicity of CD8+ T cells isolated from WT and IL-17-/- mice. If this IL-17/CD8+ T cell axis is confirmed, the mechanism by which the IL-17 stimulated CD8+ T cells combat WNV can be studied further. These findings will aid in further understanding of the pathogenesis of WNV and other viruses which may inform advances in treatments and other therapeutics, as well as in vaccine technology.

Chapter 3: Methodology

3.1 Mice and Infection

WT and IL-17-/- mice (C57BL/6) were challenged with WNV isolate 2471 in an animal Biosafety Level 3 (BSL3) facility by BSL3 certified personnel. The virus stock preparation and a subsequent plaque assay in Vero cells were used to determine the titer of the virus. All animals were handled in a BSL3 animal facility in accordance with protocols approved by the University of Southern Mississippi Institutional Animal Care & Use Committee.

3.2 Survival Analysis

WT and IL-17-/- mice (7 week, sex matched) were challenged via intraperitoneal (i.p) injection of 1000 plaque forming units (PFU) of WNV in 100 µl of PBS with 1% gelatin. Mice are observed twice daily for mortality and morbidity up to 21 days postinfection (p.i.). This survival study was also performed using a footpad infection route.

3.3 Quantitation of Viral Load

After day 4 and day 6 p.i. with WNV infection, blood and brain specimens were obtained from WT and IL17-/- mice. Total RNA was extracted by using RNeasy Mini Kit (Quigen) and complementary DNA (cDNA) was prepared using iScript cDNA synthesis kit (Biorad). *WNV-E* and cellular *β- actin* gene RNA copy numbers were measured by quantitative polymerase chain reaction (qPCR) using SYBR Green Supermix (Biorad). The viral load in each specimen was expressed as ratio of *WNV-E* copy number to that of cellular *β- actin*. Primers used for qPCR were previously

described (Bai et al., 2009).

3.4 Cell Culture

MC57GL cells expressing amino acids 1 through 402 of the WNV E protein through pcDNA3.1 vector ($MC57GL_{WNV\text{-}E}$) or pcDNA3.1 vector only ($MC57GL_{vector}$), were used as a generous gift from Michael S. Diamond (Washington University in St. Louis, School of Medicine). Both $MC57GL_{WNV\text{-}E}$ and $MC57GL_{vector}$ cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 5% fetal bovine serum (FBS) and 50 μM β-mercaptoethanol.

3.5 CD8+ T cell Isolation

Mice infected with WNV (100 PFU) were sacrificed at day 10 p.i. by CO_2 inhalation and spleens were collected in RPMI media. After isolation of the splenocyte population, cells were subjected to CD8+ T cell isolation by negative selection with magnetic beads using Mouse CD8+ T Lymphocyte Enrichment Set - DM (BD Biosciences). Isolated CD8+ T cells were confirmed using CD45 and CD8α (Ly-2) antibodies and subjected to cytotoxicity testing.

3.6 Cytotoxicity Assays

The cytotoxicity of isolated CD8+ T cells was determined as described by Shrestha and Diamond (2004) with minor modifications. $MC57GL_{WNV\text{-}E}$ and $MC57GL_{vector}$ cells served as WNV specific target cells and control cells, respectively. WNV effector CD8+ T cells isolated from both WT and IL-17-/- mice were plated in 96 well plates along with $MC57GL_{WNV\text{-}E}$ or $MC57GL_{vector}$ in a 1:100 ratio and tested for their ability to lyse the target cells. The lysis of the target cells was detected using

Cytotoxicity Detection Kit (LDH) according to the instructions of the manufacturer (Thermo Scientific). This method determined the percentage of cell lysis by colorimetric measurement of lactate dehydrogenase (LDH) released by the lysed cells.

3.7 Data Analysis

Data collected were organized and maintained in standard excel spreadsheets and analyzed using Prism 6 software (GraphPad). ANOVA and t-test were also used whenever applicable. A value of $\alpha \leq 0.05$ was assumed to be significant. Survival data were analyzed using the Kaplan-Meier survival and log-rank test (GraphPad Prism software, version 6.0). The significant variation in cytotoxicity between WT and IL-17-/- mice was interpreted as an indication of CD8+ T cell involvement in an IL-17 dependent manner during WNV infection.

Chapter 4: Results

4.1 IL-17-/- Mice Are Susceptible to Lethal WNV Infection

The mortality of WT and IL-17 -/- mice when infected with WNV was compared using both i.p. and footpad infection routes. Survival rates were significantly lower in IL-17 -/- animals than in WT for both injection routes (Fig. 4.1A and B). About 60% of WT mice survived WNV challenge via i.p. injection compared to about 20% of IL-17 -/- mice. Similarly, in footpad inoculation, about 60% of WT mice survived compared to about 10% of IL-17 -/- mice. Collectively, these data suggest that IL-17 plays a protective role during WNV infection.

A. B.

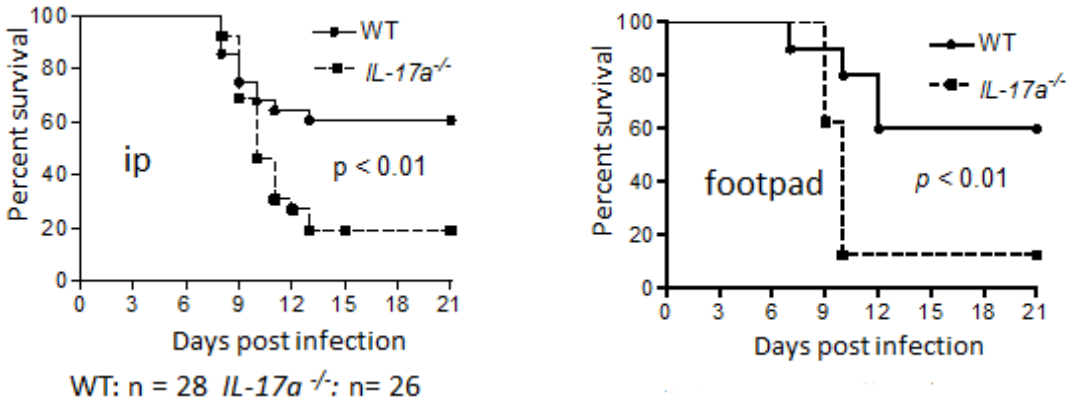

Figure 4.1. Survival analysis for WT and IL-17 -/- mice inoculated with WNV. Mice (7 weeks old, sex matched) were infected with 1000 PFU of WNV and monitored twice daily for survival. Survival data were analyzed by log rank test. (A) Survival data of WT and IL-17 -/- mice inoculated via i.p. injection. (B) Survival data of WT and IL-17 -/- mice inoculated via footpad injection.

4.2 IL-17-/- Mice Develop Increased Viral Load in Blood and Brain

After WNV infection, viral loads in the blood and brain of both WT and IL-17-/- mice were measured using qPCR. At day four, the viral load in the blood of IL-17-/- mice was much higher than that in the WT mice (Fig.4.2A). Similarly, the viral load in the brain at day seven was much higher in IL-17-/- mice than in WT mice (Fig. 4.2B). These data suggest that IL-17 plays an important role in controlling both viremia and WNV replication in brain.

A. B.

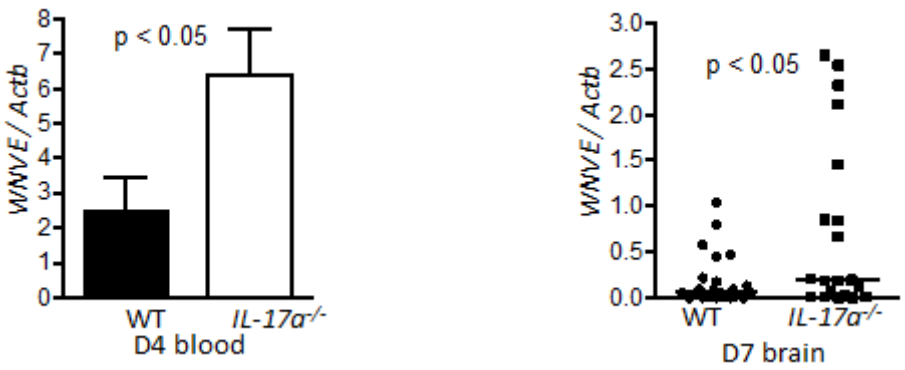

Figure 4.2. IL-17-/- mice produce high viral load in blood and brain. WT and IL-17-/- mice were infected with 1000 PFU of WNV. (**A)** Blood collected at day 4 was analyzed for WNV RNA copy number by qPCR. (**B)** Viral load in brain was analyzed at day 7 by qPCR.

4.3 Cytotoxicity of CD8+ T cells

Previous studies have shown that CD8+ T cells play an important role in clearance of WNV and protection from fatal WNV infection. In order to assess the effect of IL-17 on CD8+ T cells, the cytotoxicity of WNV primed CD8+ T cells from WT and

IL-17-/- mice was tested against target cells expressing WNV E protein. A significant reduction in killing of WNV specific target cells ($MC57GL_{WNV\ E}$) by CD8+ T cells isolated from IL-17-/- mice was detected when compared to WT CD8+ T cells (Fig. 4.4). As expected, WT and IL-17-/- CD8+ T cells had approximately the same level of cytotoxicity against control cells ($MC57GL_{vector}$). These results suggest that IL-17 plays an important role in CD8+ T cell mediated clearance of WNV.

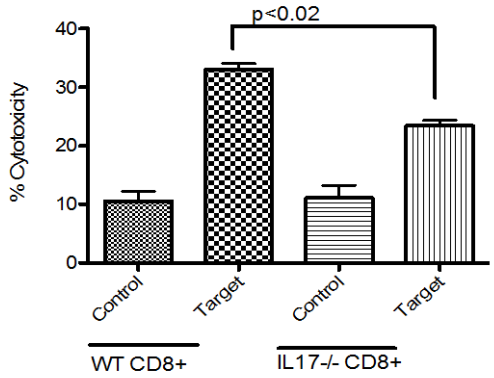

Figure 4.3. Cytotoxicity of WT and IL-17-/- CD8+ T cells. WT and IL-17-/- mice were infected with 100 PFU of WNV through i.p. route. Spleens were collected at day 10 post infection and subjected to CD8+ T cell isolation using magnetic beads. Purified CD8+ T cells were mixed with WNV specific target ($MC57GL_{WNV\ E}$) or control ($MC57GL_{vector}$) cells in 1: 100 ratios (target to effector) and incubated at 37˚C. After 4 hours of incubation, the lactate dehydrogenase (LDH) released from lysed cells was detected using an LDH cytotoxicity detection kit and the percentage of cytotoxicity was calculated.

Chapter 5: Discussion

The proinflammatory cytokine IL-17 is known to play an important role in development of neutrophils and their recruitment to infection sites, and has been linked to several autoimmune and inflammatory diseases (Korn et al., 2009; Nakae et al., 2003; Langrish et al., 2005). In addition, IL-17 has been implicated in several microbial infections including *Klebsiella pneumoniae*, *Bordetella pertussis*, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis*, and *Candida albicans* (Korn et al., 2009) and in inflammation of the CNS, including experimental autoimmune encephalitis (Komiyama et al., 2006), but its role during WNV infection has not been studied before. Cytokine interleukin-23 (IL-23) plays an important role in the development of Th17 cells, a major cell type producing IL-17. It is previously shown that an IL-23 response is induced during WNV infection via a Toll-like receptor 7 (TLR7) dependent pathway (Town et al., 2008), suggesting that IL-17 might play a role in the immune response to WNV. This study aimed to assess whether IL-17 plays a role during WNV immunity. The survival of IL-17 -/- mice studied against WT mice in response to lethal WNV challenge showed that the number of mice that survived after WNV challenge was much lower in the IL-17-/- compared to that of the WT mice (Fig. 4.1A and B). Consistent with reduced survival, viral load in the blood and brain of IL-17-/- mice was significantly higher when compared to WNV infected WT mice (Fig. 4.2A and B). The increased viral loads found in IL-17-/- mice indicate that IL-17-/- mice are less capable of controlling WNV, suggesting a protective role of IL-17 during WNV infection. Supporting this result, IL-17 is known to play a protective role during several other viral infections including influenza and vaccinia viruses (Hamada et al., 2009; Yeh et al., 2010). A

protective role of IL-17 in several fungal and bacterial infections has been previously reviewed, as well (Curtis & Way, 2009; Bar et al., 2014).

It is previously reported that cytotoxic CD8+ T cells play an important role in clearance of WNV infection (Shrestha & Diamond, 2004; Wang et al., 2003). The primary methods of killing utilized by the CD8+ T cells are both a perforin dependent mechanism and a Fas-FasL interaction, both of which result in apoptosis of WNV infected cells (Shrestha et al., 2006; Shrestha & Diamond, 2007). However, the mechanism of CD8+ T cell activation and cytoxicity during WNV infection is not clearly understood. This study tested the possible role of IL-17 in CD8+ T cell function during WNV infection by subjecting CD8+ T cells collected from WNV infected IL-17-/- mice to a cytotoxicity assay. The cytotoxicity assay showed a reduced level of cytotoxicity in the CD8+ T cells isolated from IL-17-/- mice compared to WT CD8+ T cells (Fig. 4.4). The reduced cytotoxicity exhibited by the IL-17-/- CD8+ T cells indicates that IL-17 plays a role in the cytotoxic ability of the CD8+ T cells. Based on these findings, it follows that IL-17 produced during WNV infection might play a role in the CD8+ T cell mediated clearance of the virus. Although the role of IL-17 in CD8+ T cell cytotoxicity was not previously understood, the possible connection between CD8+ T cells and IL-17 had been suggested (Billerbeck et al., 2010; Yeh et al., 2010; Xu et al., 2010). A population of CD8+ T cells called Tc17, which produce IL-17, has been shown to provide protection during influenza virus infection (Hamada et al., 2009), vaccinia virus infection (Yeh et al., 2010) and hepatitis C virus infection (Billerbeck et al., 2010). Similarly, IL-17 produced by γδ T cells has been found to promote CD8+ T cell activation during infection with the intracellular bacteria *L. monocytogenes* (Xu et al.,

2010).

In conclusion, this study suggests that IL-17 plays an important role in the immune response to WNV infection. IL-17 is likely produced in response to IL-23 after detection of viral ssRNA by TLR7 and contributes to the cytotoxicity of CD8+ T cells during the adaptive immune response. This result provides a novel role of IL-17 in the adaptive immune response to WNV infections. In future studies, the precise mechanism by which IL-17 controls the cytotoxicity of CD8+ T cells will need to be elucidated. Since IL-17 can be produced by many different cell types and might possibly regulate CD8+ T cell biology at many different stages including development and function, further research is warranted for a complete understanding of the role of IL-17 in the immune response to WNV infection.

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