

## Research Highlight

# A Little ‘Help’ from IL-21 During Persistent Viral Infection

Dorian B. McGavern\*

National Institute of Neurological Disorders and Stroke, The National Institutes of Health, Bethesda, MD 20892, USA

\* Correspondence to: Dorian B. McGavern, Tel: +1 301 443 7949; E-mail: mcgavernd@mail.nih.gov

**Anti-viral CD4<sup>+</sup> T cells are required to orchestrate and sustain the activities of the adaptive immune system during a persistent viral infection. Three recent studies suggest that CD4<sup>+</sup> T cells accomplish this in part through the release of IL-21.**

Despite decades of intense research, persistent viral infections still continue to outsmart investigators and maintain an active presence in the human population. Recent estimates suggest that ~8–12 chronic infections can be found in each of us and comprise what is referred to as our ‘virome’ (Virgin et al., 2009). For better or for worse, persistent viral infections do influence human physiology. At one end of the spectrum, persistent infections can heighten immune awareness, helping to ward off other pathogenic invaders seeking a new home in which to replicate. This is considered advantageous, as occupation by a benign infection is better than invasion by a pathogenic one. Pathogenic persistent viral infections lie at the other end of the spectrum and are, for good reason, the focus of major investigations within the research community. Viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), papilloma virus, and adenovirus can negatively impact human health by causing well-described symptoms and chronic diseases. It is because of these diseases that researchers are determined to understand every facet of their biology as well as how our immune system senses and responds to these pathogens. The fact that these pathogens persist signifies that they have already achieved the upper hand, and thus researchers face the challenge of how to tip the balance back in favor of our immune system. This is particularly

difficult when dealing with pathogens that establish latency such as HIV, EBV, CMV and papilloma virus. These viruses have the ability to lie dormant, avoiding detection by the immune system.

A strategy commonly used in the research community to gain novel insights into our anti-viral defenses is to study murine models of persistent viral infection. Although mouse and human are by no means identical, enough similarities exist such that fundamental immunological discoveries which apply to humans are routinely uncovered in murine systems. One of the most widely used murine viral model systems relies on a pathogen known as lymphocytic choriomeningitis virus (LCMV). Some of the most seminal findings in viral immunology, such as the mapping of MHC I restriction, were discovered using this model system. A major advantage of working with the LCMV model is the availability of advanced tools and well-characterized strains of the virus that yield unique outcomes upon inoculation. Two commonly used paradigms in the field involve the use of LCMV Armstrong/Clone 13 (Ahmed et al., 1984) and LCMV Aggressive/Docile (both derived from LCMV WE) (Pfau et al., 1982). Injection of high-dose ( $2 \times 10^6$  PFU) Clone 13 or Docile results in a protracted phase of viral clearance, whereas LCMV Armstrong and Aggressive injected in a similar manner are purged acutely (i.e. 7–10 days).

The Armstrong/Clone 13 comparison has been particularly useful in identifying

factors associated with protracted clearance. For example, it is well described that intravenous inoculation with high-dose Clone 13 is associated with functional exhaustion/deletion of LCMV-specific CD8<sup>+</sup> (Zajac et al., 1998) and CD4<sup>+</sup> T cells (Brooks et al., 2005), which is not observed in mice infected with Armstrong. Exhaustion is defined as a reduced ability (or inability) of anti-viral T cells to produce certain cytokines and effector molecules. Importantly, similar exhausted anti-viral T cells have been found in patients persistently infected with HIV and HCV, suggesting an association between viruses that persist and T cell exhaustion. Because of this association, it was surmised that reversal of the exhaustive state might promote viral clearance. In the Clone 13 model, three molecules [PD-1 (Barber et al., 2006), IL-10 (Brooks et al., 2006) and LAG-3 (Blackburn et al., 2009)] were recently shown to be up-regulated during the chronic stage of infection, and blockade (or genetic deletion) of these molecules facilitates viral clearance. Blockade of PD-1 also improved anti-viral responses in primates infected with simian immunodeficiency virus (Velu et al., 2009), suggesting that interference with inhibitory molecules might prove useful for the treatment of persistent viral infections in humans such as HIV infection.

The LCMV model has undoubtedly proven useful for the identification of factors associated with persistence that apply to chronic viral infections in

humans. Another fundamental feature of the LCMV model is the requirement for anti-viral CD4<sup>+</sup> T cells in the maintenance of functional CD8<sup>+</sup> T cells and the eventual clearance of the virus (Matloubian et al., 1994). Over a period of months, LCMV Clone 13 is eventually controlled in immunocompetent hosts; however, in the absence of CD4<sup>+</sup> T cells, the virus persists at high titers indefinitely. It is presently not known how anti-viral CD4<sup>+</sup> T cells sustain the activities of CD8<sup>+</sup> T cells in the LCMV model, but three papers recently published in *Science* have shed light on this important question by identifying a cytokine (IL-21) produced by anti-viral CD4<sup>+</sup> T cells that acts on CD8<sup>+</sup> T cells and is required for the clearance of immunosuppressive LCMV strains (i.e. Clone 13 and Docile) (Elsaesser et al., 2009; Frohlich et al., 2009; Yi et al., 2009).

IL-21 is a member of a family of cytokines that bind to the common cytokine receptor  $\gamma$  chain (Spolski and Leonard, 2008). This family includes IL-2, IL-4, IL-7, IL-9 and IL-15. The receptor for IL-21 is expressed on T cells, B cells, NK cells, dendritic cells, macrophages, epithelial cells and keratinocytes, and the cytokine itself is produced predominantly by CD4 T cells (Th1, Th2 and Th17 lineages). Fitting with its diverse receptor expression pattern, IL-21 is thought to play a role in CD4<sup>+</sup> T cell differentiation/function, NK cell cytotoxicity/proliferation, plasma cell differentiation, immunoglobulin production and cytotoxic lymphocyte function/proliferation/survival. In fact, IL-21 is presently being tested in clinical trials for the treatment of certain types of cancer. The diversity of immune functions associated with IL-21, and its production predominantly by CD4<sup>+</sup> T cells, made it an interesting candidate to consider during a persistent viral infection.

In these three recently published studies, investigators used the LCMV Clone 13 (Elsaesser et al., 2009; Yi et al., 2009) and Docile (Frohlich et al., 2009) models of chronic infection to define the importance of IL-21 in facilitating anti-viral immunity. IL-21 was found to be produced predominantly by LCMV-specific CD4 T cells at both early and late time points post-infection. Interestingly, infection of IL-21 receptor knockout mice with the

immunosuppressive variants of LCMV resulted in more pronounced CD8<sup>+</sup> T cell exhaustion/deletion and an inability to eventually control the infection. In contrast, IL-21 receptor-knockout mice had no difficulty clearing LCMV Armstrong or WE, which are purged acutely following intravenous inoculation. Virus-specific T cells expanded normally, and a memory T cell pool emerged following LCMV clearance. It was postulated in all three studies that IL-21 acts directly on anti-viral CD8<sup>+</sup> T cells during chronic infection, and this was based on two lines of investigation. First, infection of bone marrow chimeras containing wild-type and IL-21 receptor knockout bone marrow revealed that wild-type LCMV-specific CD8<sup>+</sup> T cells numerically out-competed their receptor knockout counterparts. Second, direct injection of IL-21 into CD4<sup>+</sup> T cell knockout mice, which normally show heightened exhaustion following Clone 13 infection, restored some function to anti-viral CD8<sup>+</sup> T cells and reduced viral loads. Unfortunately, treatment was also associated with 70% mortality, suggesting that direct injection of IL-21 promotes intolerable levels of immunopathology during chronic infection. This result needs to be taken into consideration when considering IL-21 as a therapy for persistently infected humans.

In addition to the T cell defects, a significant reduction in LCMV-specific antibodies was also observed in IL-21 receptor knockout mice following infection with immunosuppressive LCMV strains, consistent with the importance of IL-21 in the generation of plasma cells and production of immunoglobulins (Spolski and Leonard, 2008). Although the anti-viral B cell response was not a major focus of these studies, it is important to consider the collective impact of IL-21 on adaptive immunity in the Clone 13 and Docile models. Recent studies have shown that an antibody response is important for controlling chronic LCMV infections (Bergthaler et al., 2009), and IL-21 receptor deficiency on B cells could strain CD8 T cell responses by impeding antibody production and consequently elevating viral titers. CD8 T cells exposed to higher viral loads would be expected to exhaust more quickly, which is the phenotype observed in IL-21 receptor knockout mice. Additional studies are

required to tease out the exact role played by IL-21 on anti-viral B cells versus CD8<sup>+</sup> T cells during chronic viral infection.

Given the complexity and diversity of persistent viral infections, it is important to understand all of the factors that decide the outcome of a viral infection. Following infection, many factors can dictate whether a virus transitions into a state of persistence. These recent studies indicate that IL-21, a cytokine produced by anti-viral CD4 T cells, is one such factor that orchestrates successful viral clearance following LCMV infection. Because IL-21 has the capacity to impact multiple arms of the immune system simultaneously, an important next step will be to define how IL-21 influences each immune cell that expresses its receptor during chronic infection. It will also be important to extend these findings to persistent viral infections in humans.

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