# Cytokine-mediated regulation of immunity during persistent viral infection

Pratumchai, I.

# Chapter 1

## General Introduction

**Persistent viral infection and Lymphocytic Murine Choriomeningitis Virus as a model system**

Chronic viral infections such as human immunodeficiency virus (HIV), Hepatitis C virus (HCV) and hepatitis B virus (HBV) are a major global health issue with hundreds of millions of people infected worldwide(1). HIV infection, which leads to acquired immunodeficiency syndrome (AIDS), has caused more than 35 million deaths globally(2). Although potent antiretroviral therapy has successfully saved many lives in the past decade, the emergence of HIV drug resistance remains a public health concern(3). HBV and HCV can cause serious liver infections that put infected people at high risk of liver cirrhosis and cancer(4, 5). Despite tremendous disease burden caused, effective vaccine against HCV is currently unavailable(4). Chronic viral infections are characterized by prolonged viremia, sustained immune activation, disorganized splenic microarchitecture, upregulation of suppressive immunoregulators, and dysfunctional virus-specific T and B cells(6). Since viruses employ different mechanisms to manipulate and escape host immune response, sophisticated cellular interactions within the immunological network are critical to purge the infection. Better understanding of how the immune system respond to persistent viral infections will allow us to determine the pathological consequences of infections and identify effective immunotherapeutic approaches to treat the infections as well as minimize the spread and emergence of drug-resistant virus.

Animal models of persistent viral infection, such as Lymphocytic choriomeningitis virus (LCMV) Clone 13 (Cl-13), has become a critical tool for uncovering fundamental concepts that revolutionize and shape the field of immunology(7). LCMV is an Old World Arenavirus that infects rodents and can be transmitted to humans via saliva, droppings and urine of infected animals(8-11). LCMV genome consists of short (S, approximately 3.5kb) and long (L, approximately 7.2kb) single-stranded RNA segments(12). Infection with LCMV can lead to lymphocytosis and meningeal inflammation especially in immunocompromised individuals and can lead to congenital hydrocephalus and mental retardation in fetuses if infected during pregnancy(13-16). One of the great advantages of LCMV for studying immune responses and determining the cytotoxicity of immune cells is the noncytolytic property, meaning clinical disease and tissue injuries during infection are exclusively caused by the host immune reaction. In addition, different strains and routes of LCMV infection result in wide spectrum of immune responses, allowing the model to be widely used to accurately study pathogenic outcomes of the infection(7). Since the discovery of LCMV in 1934 (17), it has proven to be an invaluable experimental model that contributed to many advancements of our understanding of cellular and humoral immunity. Notably, the use of LCMV has led to the award of the 1996 Nobel Prize in Physiology and Medicine to Peter Doherty and Rolf Zinkernagel for the seminal discovery of major histocompatibility complex (MHC)-restriction of T cell recognition made between 1973-1975 (18, 19). Prior to this discovery, immunological compatibility was a concept studied in transplantation where T lymphocytes could kill foreign cells after recognizing strain-specific membrane proteins differentially expressed on cells of different mouse strains known as MHC antigens. During their studies using LCMV, Zinkernagel and Doherty revealed that T cells must simultaneously recognize both foreign and self MHC antigens to kill infected cells. The key experiment was performed by mixing LCMV specific cytotoxic T lymphocytes (CTL) derived from mice with different (histocompatibility-2) H-2 haplotypes with target cells which were LCMV-infected mouse fibroblasts (L929) derived from CBA/H mice with an $H\text{-}2^{k/k}$ allele. They examined the ability of CTL to kill target cells and observed that CTL derived from mice with at least one allele that is H-2k could specifically killed infected L929 cells(20). This finding further

allowed us to understand that once foreign peptide is processed, MHC molecules bind to peptide fragments and present them to T cells in a form of peptide-MHC complex (pMHC). T-cells receptors (TCRs) then recognize pMHC and mediate killings of infected cells. This paradigm-shifting discovery has advanced our knowledge of how antigen-specific T cells recognize virus infected cells and enabled us to develop more tools such as tetramers and TCR transgenic mice that made LCMV an invaluable system for studying cellular immune response during persistent viral infection(21). Remarkably, MHC tetramers have been widely used to accurately detect and measure functions of antigen-specific T cells. The design and utilization of tetramer was first described in 1996 in the laboratory of Mark Davis(22). To generate MHC tetramers, a known epitope is loaded on an MHC molecule of the allele that most optimally presents the epitopes to create pMHC. Then, the four loaded soluble biotinylated pMHC were mixed with fluorochrome-conjugated streptavidin to create tetramerization of MHC monomers that can stably bind to TCRs. After staining with MHC tetramer, antigen-specific T cells can then be evaluated using standard flow cytometry.

Given that virus-specific T cells are essential for antiviral immunity, TCR transgenic (Tg) mice with high frequencies of certain TCRs were developed(23). This allows a large number of antigen-specific T cells to be obtained and used to gain in-depth understanding of how antigen-specific T cells regulate antiviral immunity. The two commonly used TCR Tg mice to study T cell responses during LCMV infections are P14 and SMARTA TCR Tg mice. P14 TCR Tg mice have CD8 T cells repertoire that expresses a rearranged TCR (Tcra-V2, Tcra-J TA31 / Tcrb-V8.1, Tcrb-D, Tcrb-J 2.4) specifically recognizing MHC-I-specific LCMV glycoprotein (GP) 33–41 peptide and do not develop endogenous mature TCR alpha beta cells(23). P14 TCR Tg mice enabled scientists to study CD8 T effector functions and memory formation during acute LCMV infection as well as T cell exhaustion and viral pathogenesis during persistent LCMV infection(24). Similar to P14 mice, SMARTA TCR Tg mice do not develop endogenous mature TCR alpha beta cells but have CD4 T cells repertoire that expresses a rearranged TCR (Vα2.3-JαDK1 and Vβ8.3-Jβ2.5) specifically recognizing LCMV glycoprotein (GP) 61–80 H2-Ab complexes(25). SMARTA TCR Tg mice are used to study the role of virus specific CD4 T cells in supporting CD8 T cells functions and promoting viral clearance.

The original clone of LCMV, which is LCMV Armstrong (LCMV-Arm), was isolated in 1934 by Charles Armstrong, whom the virus was named after(17). In 1984, several mutant strains of LCMV-Armstrong were isolated by Rafi Ahmed, a postdoctoral fellow in the Oldstone laboratory(26). One of the clones, Arm53b, was isolated and observed to causes acute infection with severe inflammation in the meninges that can be resolved within 10 days. On the contrary, clone number 13 (LCMV Cl-13) can establish persistent infection that last up to 3 months despite having only 3 amino acids different from those of LCMV-Arm(26). The ability of LCMV Cl-13 to establish persistent infections is governed by two amino acids, one phenylalanine to leucine mutation at position 260 of the viral glycoprotein (GP260) and another lysine to glutamine mutation within the polymerase (L1079)(27). The third asparagine to aspartic acid mutation of GP176 is not critical for viral persistence(27, 28). Mechanistically, mutation of GP260 contributes to viral persistence by increasing receptor binding affinity causing LCMV Cl-13 to infect larger number and wider range of cells including DCs and macrophages. Infected DCs exhibit reduced expression of MHC I, MHC II, CD40 and CD80c causing DCs to be less efficient at presenting antigens and inducing T cell activation. Unlike GP260 mutation, mutation of L1079 contributes to viral persistence by increasing viral load and prolonging the infection(27-32), causing T cell exhaustion.

T cell exhaustion is a phenomenon reported in chronic viral infections as well as in cancer where antigen-specific CD8 T cells lose their effector functions in a hierarchical manner(6, 33-35). At early stage of T cell exhaustion, the loss of interleukin (IL)-2 is apparent before a subsequent loss of Tumor Necrosis Factor alpha (TNFα) is observed at the intermediate stage. As antigen loads remain high, T cells enter the severe stage of exhaustion where antigen-specific T cells lose their ability to produce interferon-γ (IFN-γ) as well as beta-chemokines(36-38). In addition, exhausted T cells display reduced cytolytic functions. Together, these events lead to the final stage of exhaustion where exhausted T cells ultimately undergo apoptosis and physical deletion causing dramatic reduction in the number of virus-specific T cells during viral persistence(35, 38). Another key characteristic of T cell exhaustion is increased expression of negative immunoregulator such as IL-10 and inhibitory receptors (IRs) such as programmed cell death protein 1 (PD-1), cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), T cell immunoglobulin and mucin domain 3 (Tim-3), Lymphocyte-activation gene (LAG-3), CD160, and 2B4(39-45). IRs are checkpoint proteins that regulate activating receptors in T cells. Increased expression of IRs constrains T cell functions and causes T cell exhaustion. Immune checkpoint blockades using monoclonal antibodies against CTLA-4 and PD-1 have been proven successful not only in restoring T cell functions but also in purging chronic viral infection as well as controlling cancers(39, 46). The success of immunotherapies targeting PD-1 and CTLA-4 led to the award of the 2018 Nobel Prize in Physiology and Medicine to James Allison for the discovery of CTLA-4 and Tasuko Honjo for the discovery of PD-1(46-52). After the discovery of CTLA-4 in 1978, Allison revealed the inhibitory effect it has on T cell functions and developed blocking antibody against CTLA-4. In a mouse model of colon carcinoma, blocking CTLA-4 increased tumor rejection, suggesting that immune checkpoint inhibition can be used as a therapeutic strategy for cancer treatment. Moreover, a significant advancement in immunotherapy was made when the role of PD-1 in promoting T cell exhaustion was described using LCMV Cl-13 by Daniel Barber, a postdoctoral fellow in the laboratory of Rafi Ahmed(39). Baber showed that exhausted T cells upregulate PD-1 during persistent LCMV infection. By blocking PD-1/PD-L1, the group were able to reverse T cell exhaustion and significantly accelerate viral clearance. Following these observations, multiple groups reported that PD-1 expression is associated with T cell exhaustion in HIV infected patients(53-56). Subsequent study demonstrated that blocking PD-1 during SIV infected non-human primates improved virus-specific CD8 T cell response, promoted memory B cell proliferation, enhanced SIV-specific antibody production, decreased viral load, and improved survival of infected animals(57, 58). Similar observation has been made in HCV infected non-human primates(59-61), suggesting that PD-1 blockade could potentially be an immunotherapeutic strategy for controlling T cell exhaustion in human viral infections and possibly cancers. Although the use of PD-1 checkpoint blockade is currently being assessed in human chronic viral infection, the success in using anti-PD-1 immunotherapy in treating cancers has been clinically proven(62). Taken together, these observations demonstrate that LCMV Cl-13 has been a powerful tool that enables us to understand the concept of MHC restriction, T cell exhaustion and immunotherapy. Despite the success of immune checkpoint inhibitors (ICIs) in revolutionizing the practice of medical oncology, treatment failure, resistance and relapse are frequently observed(63, 64). More studies are hence needed to improve our ability to better predict potential responders and develop new combination therapies for those who do not respond to standard ICI treatments. Recent findings identified a subset of stem-like virus-specific $TCF1^{+}$ $Tim3^{-}$ CD8 T cells that gives rise to the proliferative burst during anti-PD1/PD-L1 immunotherapy(41, 65). New immunotherapeutic

approaches targeting this subset of CD8 T cells combines with traditional ICI treatments could potentially increase the number of patients responding to ICIs.

## Cytokine signaling in viral infection and their potential for clinical therapy

Host-derived immune regulators such as cytokines are important immunomodulators that have crucial roles during persistent viral infection. Cytokines are soluble proteins that mediate cell-to-cell communication and interaction to facilitate critical stimulatory or suppressive effects, which in turn result in preventing or promoting viral persistence. Significant research efforts from both humans and mice have identified multiple cytokines that play critical roles during persistent viral infections(66, 67).

### IL-10 signaling

Interleukin-10 (IL-10) is an immunosuppressive cytokine that contributes to viral persistence(68, 69). It is produced by several immune cells including dendritic cells (DCs), monocytes, macrophages, B cells, CD4 T cells, CD8 T cells and T regulatory T cells shortly after infection(70, 71). IL-10 signals through a heterodimeric type II cytokine receptor composed of IL-10R1 and IL-10R2 subunits to activate the janus tyrosine kinases JAK1 and Tyk2 and the signal transducers and activators of transcription STAT1, STAT3, and STAT5 (72-75). Elevated IL-10 level was observed in patients with chronic viral infections including EBV, HBV, HCV, and HIV (76-80). Blockade of IL-10 resulted in increased proliferation and cytokine secretion of T cells isolated from HIV And HCVs. Similar phenomenon was observed in mice infected with persistent LCMV Cl-13 (68, 81). Faster clearance of persistent LCMV infection was observed in IL-10 deficient mice as well as in mice treated with anti-IL-10R-specific antibodies at early-stage post-infection (p.i.)(68). These mice displayed improved functions of CD4 and CD8 T cells as well as faster clearance. Mechanistically, IL-10 exerts its immunosuppressive effects on T cells by altering DC maturation via reducing the expression of the co-stimulatory molecules CD80 and MHC class I and II(69, 75). Consequently, IL-10-primed DCs exhibit reduced ability to prime CD4 Th1 cells causing CD4 T cells to become suppressive IL-10 producing cells instead of being IFNγ-producing Th1 cells. A recent study demonstrated that co-treatments of anti-IL-10R and anti-PD-L1 antibodies further enhanced T cell functions and accelerated viral clearance in mice infected with persistent LCMV Cl-13 (82). Given that IL-10 plays important roles during persistent viral infection, further understanding of the IL-10 signaling network will improve our fundamental knowledge of persistent viral infection and potentially lead to therapeutic strategies that can be used to treat chronic viral infections in human.

### IL-2 signaling

As host immune system fails to control the infection and antigen levels subsequently increase, CD8 T cells start to express inhibitory receptors such as PD-1, Lag-3, and Tim-3 before gradually losing their effector functions in a process known as T cell exhaustion. Several cytokines have been shown to positively regulate antiviral CD8 T cells and promote viral clearance(67). IL-2 is a member of a $\gamma_c$-dependent cytokine family. It was identified as a powerful growth factor for T cells and was the first cytokine to be molecularly cloned after its discovery in 1983(83, 84). During viral infection, IL-2 is primarily produced by CD8 and CD4 T cells and binds to a receptor complex composed of IL-2Rα (CD25), IL-2β (CD122), and $\gamma_c$ (CD132) subunits(85-87). Significant

findings using a mouse model of persistent LCMV infection showed that IL-2 signaling is essential for the maintenance of virus-specific CD8 T cells and administration of IL-2 improved CD8 T cell response while promoting faster control of the virus(88). Intriguingly, a combination of IL-2 administration with PD-L1 blockade substantially enhanced expansion of antiviral CD8 T cells and reduced viral load(89), suggesting that IL-2 could potentially be used as treatment for persistent viral infections in human. However, several clinical trials in HIV patients revealed that IL-2 administration was able to increase numbers of CD4 but unable to reduce viral load(90). Together, these findings suggest that even though IL-2 is a promising immunotherapeutic cytokine to treat viral persistence, better understanding of the biology of IL-2 is required to achieve the optimal therapeutic outcome.

**IL-7 signaling**

Another cytokine from a $\gamma_c$-dependent cytokine family that positively regulates CD8 T cells during chronic viral infection is IL-7. Previous studies have shown that IL-7 plays a key role during development, maintenance, and survival of T cells(91-94). IL-7 is largely produced by stromal cells and signals through a dimeric receptor complex consisting of IL-7α (CD127) and $\gamma_c$ (CD132) subunits to activates JAK1, JAK3 and STAT5(95). Recent studies demonstrated that administration of IL-7 during chronic LCMV infection promotes expansion of virus-specific CD8 T cells as well as increased numbers of CD4, CD8 and B cells causing faster clearance of the virus(96, 97). The unique capacity of IL-7 to improve host immune response and promote clearance of persistent viral infection was further explored during HIV and simian immunodeficiency virus (SIV) infections. Concomitantly, IL-7 administration in patients infected with HIV and primates infected with SIV led to increase of circulating CD4 and CD8 T cells(98, 99). Taken together, these findings uncovered that IL-7 is another cytokine with promising therapeutic effects that could potentially be used to treat persistent viral infection.

**IL-21 signaling**

Besides IL-2 and IL-7, studies have recently identified IL-21, another member of the $\gamma_c$-dependent cytokine family, as a powerful positive immunoregulator. IL-21 is mainly produced by different subsets of CD4 T cells and signals through a heterodimeric receptor complex consisting of the γc (CD132) and an IL-21-receptor (IL21R) (CD360) subunits(100-102). It signals on CD4 T cells, CD8 T cells, B cells and natural killer (NK) cells to activate JAK1, JAK3, STAT1, STAT3 and STAT5(103). Remarkable findings by three different research groups using a mouse model of persistent LCMV infection showed that IL-21R signaling is required for the maintenance of effective antiviral CD8 T cells(104-106). Mice deficient in IL-21R displayed severely impaired functions of CD8 T cells during persistent LCMV infection. Meanwhile exogenous treatment of IL-21 in chronically infected mice led to increased number and improved functions of antiviral CD8 T cells as well as reduced viral titers(106). Given the potential of IL-21 as a promising therapeutic agent for chronic viral infection, further work is required to determine the effects of IL-21 immunotherapy in different conditions and disease models to gain deeper insights on the mechanisms by which IL-21 supports effector functions of CD8 T cells and promote viral clearance. Moreover, understanding of where this vital cytokine is produced and how IL-21-producing cells are maintained will enable us to determine better therapeutic strategies to treat persistent viral infections.

**IL-6**

IL-6 is an important cytokine that supports differentiation and maintenance of Tfh cells by inducing expression of genes associated with Tfh differentiation such as Bcl-6, CXCR5 and IL-21(107, 108). IL-6 is produced mainly by follicular dendritic cells (FDC) during late stage of chronic viral infection and can also be produced by other cell types such as T cells, B cells, mast cells, macrophages, and dendritic cells in different disease models(109). IL-6 signals through a receptor complex composed of IL-6Rα and gp130, which is shared with IL-27, to activate primarily STAT3. Observations of patients infected with HIV, HBV and HCV infections revealed that reduced levels of IL-6 were associated with disease progression, suggesting IL-6 plays an important role in promoting antiviral immunity(110-112). IL-6 promotes antiviral immunity by supporting Tfh differentiation and promoting accumulation of GCB as well as production of virus-specific antibody. Consequently, IL-6 signaling during persistent viral infection leads to improved CD8 T cell functions, increased neutralizing antibody production, and faster viral control. Further study demonstrated that T cell-specific gp130 signaling is required for IL-21 production by CD4 T cells and clearance of LCMV Cl-13 infection(113). Given that IL-27 also signals through a receptor complex consisting of gp130, the authors further explored the role of IL-27 during persistent LCMV infect and uncovered that IL-27 is also required for control of LCMV Cl-13 infection and maintenance of virus-specific CD4 T cells(113).

**IL-27 signaling**

IL-27 is a heterodimeric cytokine in the IL-6 and IL-12 family composed of the IL-27p28 and Epstein Barr-Virus Inducible protein-3 (EBI3) subunits(114). It signals through a heterodimeric receptor consisting of WSX-1 and gp130, which is shared with IL-6 receptor complex to convey IL-27 signal through activating of STAT1 and STAT3 (115). IL-27 is produced and secreted by dendritic cells and macrophages upon stimulation with pathogen derived inflammatory signals through Toll-Like receptors (TLRs)(116-118). Expression of IL-27 receptor has been described in multiple cell types including naïve T cells, macrophages, B cells, endothelial cells, monocytes and activated dendritic cells, thus this cytokine has the potential to regulate multiple arms of the immune response. In addition, an interesting recent observation revealed that IL-27 is likely expressed by CX3CR1+ sympathetic-neuron-associated macrophages (SAMs) to promote control of obesity(119). Initial investigation of IL-27 receptor deficient mice revealed an important role for IL-27 signaling for induction of T helper type I (Th1) responses(120). Subsequent studies demonstrated a crucial role for IL-27 in eliminating intracellular pathogens(121, 122). Moreover, several reports have demonstrated a pathogenic role for IL-27 signaling in arthritis models in rats and experimental autoimmune encephalomyelitis (EAE) in mice(123-125). While the above findings demonstrate a clear role for IL-27 in promoting inflammatory responses, several studies have reported robust anti-inflammatory functions attributable to IL-27 signaling(126). The absence of IL-27 receptor leads to immune pathology due to elevated pro-inflammatory cytokines from T cells during protozoan infections(127). Similar to protozoan infection, mycobacterium tuberculosis infection of IL-27R KO mice lead to increased lung and liver immune pathology(128). Further, IL-27 signaling can limit the production of IL-2 during Th1 responses however the mechanism of IL-2 regulation is still a matter of debate(129-131). Moreover IL-27 signaling has been shown to suppress Th17 differentiation and induce elevated levels of the immune suppressive cytokine, IL-10(123, 132, 133). Thus, IL-27 signaling has been associated with both pro- and anti-inflammatory functions unique to the specific pathogen being studied. However, how IL-27 signaling regulates the CD8 T cell response to viral infection and cancer remains equally

interesting and complex(134). Following acute Corona and Influenza viral infections, IL-27 attenuates anti-viral CD8 T cell responses(135, 136). However, during chronic antigen persistence and inflammation, IL-27 can also have pleiotropic effects; Following chronic infection with LCMV clone-13, IL-27 deficient mice display severe T cell dysfunction and are unable to control the infection(113). IL-27 signaling is essential for promoting survival of virus-specific CD4 T cells at later stage of infection. Reduction in frequency and total number of virus-specific CD4 T cells were observed in addition to reduced LCMV-specific IgG2a/2C in IL-27ra KO mice(113). In contrast, others have reported that IL-27 signaling promotes the expression of negative immune regulatory molecules (TIM3, PD-1 etc.) on CD8 T cells in both Cl13 infection and cancer and can be detrimental to cancer immunity(137, 138). In the context of anti-tumor responses, IL-27 has been reported to both promote (139-141)and restrain(137, 138) T cell-mediated immune control by various mechanisms. In addition, during Ovalbumin priming IL-27 also promotes IL-21 production(142), which is required for promoting CD8 T cell function, antibody production and viral clearance(104-106). Disruption of IL-27 signaling causes reduction in numbers of virus-specific CD4 T cells and LCMV-specific antibody at later stage of infection(113). Overall, the regulation of CD8 and CD4 T cell responses by IL-27 is complex, and gaps in our knowledge remain about the cellular sources of IL-27 and mechanistically how this cytokine simultaneously drives immune stimulatory and suppressive properties in T cells.

**Interferon signaling**

IFN-I were initially known for their antiviral properties during acute viral infection. Paradoxically, sustained IFN-I signaling during persistent viral infections were reported by multiples studies to cause deleterious effects to host immune response leading to immune suppression and viral persistence(143). IFN-I are a large family of cytokines consisting of the well characterized subtypes IFNα and IFNβ as well as the lesser defined subtypes IFNω, IFNε, IFNδ, IFNτ and IFNζ. After viral sensing via pathogen recognition receptors (PPRs) recognizing pathogen-associated molecular patterns (PAMPS), IFN-I production is induced with IFNα being produced largely by plasmacytoid dendritic cells (pDCs)(144). Upon secretion, IFN-I signal through a heterodimeric transmembrane receptor complex composed of Interferon Alpha And Beta Receptor Subunit 1 (IFNAR1) and Interferon Alpha And Beta Receptor Subunit 2 (IFNAR2) subunits to phosphorylate JAK1 and Tyk2 in order to activate STAT1 and STAT2(145). Upon activation, STAT1 and STAT2 can assemble with Interferon Regulatory Factor 9 (IRF9) to form a trimeric transcription factor, IFN-stimulated gene factor 3, to trigger the expression of interferon-stimulated genes (ISGs), which encodes antiviral proteins(146, 147). Despite having antiviral functions, prolonged IFN-I signaling and elevated level of ISGs during HIV and SIV infections were found to be associated with disease progression and reduced number of CD4 T cells(148-150). Similarly, persistent LCMV infection in mice resulted in prolonged IFN-I signaling that contributes to viral persistence by causing disorganized lymphoid secondary lymphoid organs, reduced MHC I and II expression in DC, as well as increased PD-L1 and IL-10 expression(151, 152). Blocking IFN-I receptor at the beginning of infection by using antibody against IFNAR1 accelerated viral clearance and improved CD4 T cell response in addition to reducing IL-10 and PD-L1 expression. Furthermore, blockade of IFNβ also improved functions of CD4 T cells and facilitated faster control of the infection when blockade of IFNα failed to exert the same effects(153). These findings suggest that IFNβ plays a dominant role in causing immune suppression during persistent viral infection when compared with IFNα. Recent studies revealed that IFN-I signaling during chronic viral infection suppresses B cells and inhibits the formation of the stem-like virus-specific

TCF1$^+$CD8 T cells that gives rise to proliferative burst after PD-1 blockade(154-157). Since IFNAR blockade resulted in increased TCF1$^+$ CD8 T cells, it would be interesting to see whether blocking IFN-I can augment the ability of anti-PD-1 immunotherapy to drive CD8 T cell proliferation and viral clearance. Overall, these observations suggest that IFN-I at early stage of infection can be deleterious to the host immune system during persistent viral infection. However, therapeutic strategies targeting IFN-I should be fine-tuned to achieve the most effective outcome with the least possible side-effects given that IFN-I also has other antiviral benefits depending on the timing and type of viral infection. In-depth understanding of these cytokines will allow us to design better immunotherapies through the usage of cytokines, blocking antibodies, or even Jak inhibitors to fight against human disease.

**Thesis Outline**

This PhD thesis focuses on understanding the biology of IFN-I and IL-27 during persistent LCMV infection. Both cytokines were shown to play crucial but opposing roles in influencing functions, expansion, and survival of virus-specific T cells(113, 151, 152). **Chapter 2** demonstrates the interplay between these two cytokines and the impact that it has on promoting expansion and survival of the stem-like TCF1$^+$CD8 T cells that were recently identified as a subset of exhausted CD8 T cells with high proliferative potential after anti-PD-1 treatment. Based on a recent discovery that blockade of type 1 IFN resulted in expansion of the stem-like TCF1$^+$ CD8 T cells(154), we demonstrate that this process happens in an IL-27- and STAT1-dependent manner. In addition, we show that IL-27 promotes expression of transcription factor Interferon Regulatory Factor 1 (IRF1) to support proliferation and promote survival of the cells. **In chapter 3**, we discover that B cells secrete IL-27 upon LCMV Cl-13 infection and that B-cell-derived IL-27 is required for the control of persistent LCMV infection. We further show that B-cell-derived IL-27 is crucial for accumulations of virus-specific T cells and Tfh cells. B-cell-derived IL-27 also induces IL-21 production by Tfh cells, which subsequently induces antibody class switch. In **chapter 4**, we explore the possibility of using Jak inhibitor ruxolitinib to rescue T cell exhaustion. We demonstrate that ruxolitinib treatment during persistent viral infection is able to increase the total number of the stem-like TCF1$^+$ CD8 T cells. In addition, ruxolitinib works in synergy with anti-PD-L1 to promote viral clearance. Further, we examine the impact of ruxolitinib in preclinical tumor model as well as in a clinical trial of Hodgkin lymphoma resistant to checkpoint inhibitors. We show that ruxolitinib positively regulates T cells and enhanced checkpoint blockade.

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