The role of sCD127 in IL-7-Mediated T cell homeostasis *in vivo*

Nawaf Aloufi

Thesis submitted to the University of Ottawa

in partial fulfillment of the requirements for the master's degree in

Microbiology and Immunology

Department of Biochemistry, Microbiology and Immunology

Faculty of Medicine

University of Ottawa

uOttawa

Abstract

Interleukin-7 is an essential cytokine that plays a major role in the development and homeostatic maintenance of T-cells. The presence of soluble forms of various cytokine receptors have been proposed to be involved in the endogenous regulation of cytokine activity. Due to the natural ability of soluble CD127 (sCD127) to bind to IL-7, there is an interest in its potential application as an immunotherapeutic agent in diseases, where IL-7 has been found to be relevant, including HIV infection. In this study, I hypothesize that by administering sCD127 to healthy mice, IL-7 activity should be enhanced, thus enhancing T cell proliferation *in vivo*.

The work presented here focuses on three main objectives: 1) evaluating the effect of IL-7 with or without sCD127 on T cell proliferation in healthy mice; 2) validating a mouse model of T cell depletion using anti-CD4 and CD8 antibodies; and 3) determining the effect of sCD127 treatment with or without IL-7 on T cell reconstitution and proliferation in the T cell depletion model. To assess the effect of administering exogenous sCD127, IL-7 or the combination on T cell proliferation, peripheral blood mononuclear cells and spleen were isolated, and stained to characterize T cell number, proliferation, and surface CD127 expression by flow cytometry. For the T cell depletion model, wild type C57BL/6 mice were injected intra-peritoneally with 150 μg single dose of anti-CD4 and anti-CD8 depleting antibodies. Consequently, mice were bled weekly to demonstrate the kinetics of T cell reconstitution following depletion (from d7 to d63).

Our results demonstrated that in healthy mice daily treatment with murine IL-7 significantly stimulated T cell proliferation and consequently increased cell number. This observation was further boosted by pre-complexing IL-7 with sCD127. For T cell depletion

experiments, the kinetics of T-cell reconstitution was different between the $CD4^+$ and $CD8^+$ T cells. $CD4^+$ T cell reconstitution was almost complete 6 weeks following T cell depletion, while $CD8^+$ T cells were only partially reconstituted at this time point. Treatment with IL-7 or combined therapy had a transient and significant effect on T cell proliferation and reconstitution, and this influence was abrogated after treatment discontinuation. Interestingly, $CD8^+$ T cells exert greater responses to our treatments in that a more pronounced proliferation and significant increase in cell number was observed relative to the effect seen on $CD4^+$ T cells in both healthy and depleted mice.

In conclusion, antibody-mediated T cell depletion is a potentially valuable tool to investigate lymphopenia-induced proliferation and potential therapies thereof. This study suggests that combining sCD127 and IL-7 therapies enhances IL-7-mediated T cell proliferation, and provides important information for the potential therapeutic use of sCD127 and its impact on IL-7 function.

Acknowledgements

First and foremost, thanks to Allah "the most merciful and gracious" for blessing me with the strength and knowledge to fulfill my goals and to accomplish this work.

I would like to extend my sincere gratitude to my supervisor and mentor, Dr. Jonathan Angel for his continued guidance, support, and encouragement throughout the years of my MSc degree. My profound appreciation to you for your patience, especially during the wailing stages of thesis writing. Working with you has been a blessing.

I would also like to thank my co-supervisor Dr. Seung-Hwan Lee for his unlimited support, valuable advice, and giving me this extraordinary opportunity to work and study in his lab.

I am particularly grateful and deeply indebted to Dr. Joanne Mcbane who has patiently proofread this thesis and for her invaluable advice, detailed and constructive comments during this thesis-writing period.

I would like to thank all of the past and present members of the Angel and the Lee lab. All of you have created a great work environment. Special thanks go to Alaa Ali and Stephanie Burke Schinkel who provided technical assistance during my studies, valuable discussion and advice.

I am very grateful to my country, the Kingdom of Saudi Arabia, represented by King Saud bin Abdulaziz University for Health Sciences (KSAU-HS) for the scholarship and financial support during the period of my study here in Canada.

I would also like to express my appreciation to all my friends in Ottawa, particularly Akram Alwithenani, Hamza Ali, Rached Daoud, and Alaa Ali for their excellent help and support to conquer the obstacles I faced during my study.

Finally, the chain of my gratitude would be definitely incomplete if I would forget to thank my wonderful parents, Abdulrahman and Aisha, for their guidance, boundless support, continuous encouragement, prayers, and infinite love. Without you, I couldn't have accomplished this, and many others. Special thanks to my sweetheart Shatha for your unconditional love, sacrifices, and support. You were with me during the whole story, I owed you a profound debt of gratitude. Many thanks go to my lovely brothers and sister for their boundless support, encouragement and prayers.

It is to them I dedicate this thesis...

Table of Contents

List of Abbreviations

ACK	Ammonium-chloride-potassium
ADAMs	A disintegrin and metalloproteinases
ADCC	Antibody-dependent cellular cytotoxicity
AKT	Protein Kinase B
ANOVA	Analysis of variance
APC	Antigen presenting cell
APC-fluoro	Allophycocyanin
B cells	B lymphocytes
BCL-2	B-cell lymphoma 2
BM	Bone marrow
B6	C57BL/6 mice
BCR	B cell receptor
BV421/BV786	Bright violet (fluorochromes)
CCR7	C-C chemokine receptor type 7
CD	Cluster of differentiation
CD62L	Cell adhesion molecule L-selectin
CD127	IL-7 Receptor alpha subunit
$CD4^+$ T cells	Helper T cells
$CD8^+$ T cells	Cytotoxic T cells
CDKs	Cyclin-dependent kinases
CLRs	C-type lectin receptors
d	day
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DN	Double-negative
DNA	Deoxyribonucleic acid
DP	Double-positive
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
H	Human
HSCs	Hematopoietic stem cells
IL-2	Interleukin 2
IL-7	Interleukin 7
IL-15	Interleukin 15
i.p.	Intraperitoneal
IFNs	Interferon genes
JAKs	Janus kinases
LAL assay	Limolus amebocyte lysate (bacterial endotoxin test)

mAbs	Monoclonal antibodies
MCL-1	Myeloid leukemia cell differentiation protein 1
MHC	Major histocompatibility complex
mins	Minute(s)
MMP	Matrix metalloproteinases
mRNA	Messenger ribonucleic acid
Ms	Mouse
NK cells	Natural killer cells
NKT cells	Natural killer T cells
NLRs	Nucleotide-binding oligomerization domain-like receptors
ns	Not significant
PAMPs	Pathogen associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffer saline
PE	Phycoerythrin
PE/Cy7	Phycoerythrin/Cyanine7
PFA	Paraformaldehyde
PI3K	Phosphatidylinositol 3-Kinase
PRRs	Pattern-recognition receptors
RAG	Recombination activating genes
RBCs	Red blood cells
Rh	Rhesus macaques
RNA	Ribonucleic acid
ROR-γt	Retinoic acid receptor-related orphan receptor-γt
ROS	Reactive oxygen species
RPMI-1640	Roswell Park Memorial Institute 1640 medium
RT	Room temperature
SB	FACS staining buffer
sCD127	Soluble IL-7 Receptor alpha subunit
SCID	Severe combined immunodeficiency
SD	Standard deviation
Sh	Sheep
SLOs	Secondary lymphoid organs
STAT	Signal Transducer and Activator of Transcription Proteins
T cells	T lymphocyte
TCR	T cell receptor
T_{cm}	Central memory T cells
T_E	Effector T cells
T_{eff}	Effector memory T cells
TLRs	Toll-like receptors

T_M	Memory T cells
T_N	Naïve T cells
TNFα	Tumor necrosis factor alpha

List of Figures

List of Tables

1. Chapter 1: Introduction

1.1 Overview of the Immune System

The immune system is defined as a collection of organs, tissues, and cells within an organism that protects against diseases mediated by pathogens (bacterial, fungi, viral, or parasitic infection) or tumors. This system has the ability to distinguish between self-antigens and non-self-antigens (1). Cells of the immune system require sophisticated mechanisms that ensure a host can specifically recognize, contain, and eventually eradicate pathogens while maintaining host integrity from uncontrolled immune activation. Traditionally, the immune system has two major aspects of immunity, known as innate and adaptive immunity, that cooperate to eliminate threats posed by pathogens to the host. The first line of defense is the innate immune response, which provides an immediate response to pathogens. The initial protective response is followed by an adaptive immune response that is more specific to the encountered antigens, and is associated with developing immunological memory.

Organs of the immune system are distributed throughout the body, and are mainly classified into primary and secondary lymphoid organs (SLOs) (**Figure 1-1**). The primary organs are the bone marrow (BM) and the thymus, which are the main sites of immune cell generation. Most of the immune cells are generated in the BM from common lymphoid or myeloid progenitors that further differentiate into distinct lineages, through a process called hematopoiesis. Unlike other immune cells, T lymphocytes (T cells) develop and mature in the thymus (2). SLOs include the spleen, lymph nodes, and mucosa-associated lymphoid tissues (MALT). MALT can be further subdivided into gut-associated lymphoid tissue (Peyer's patches, small intestine lymph nodules), bronchus-associated lymphoid tissue, tonsils, adenoids ("Waldeyer's ring"), and appendix (3).

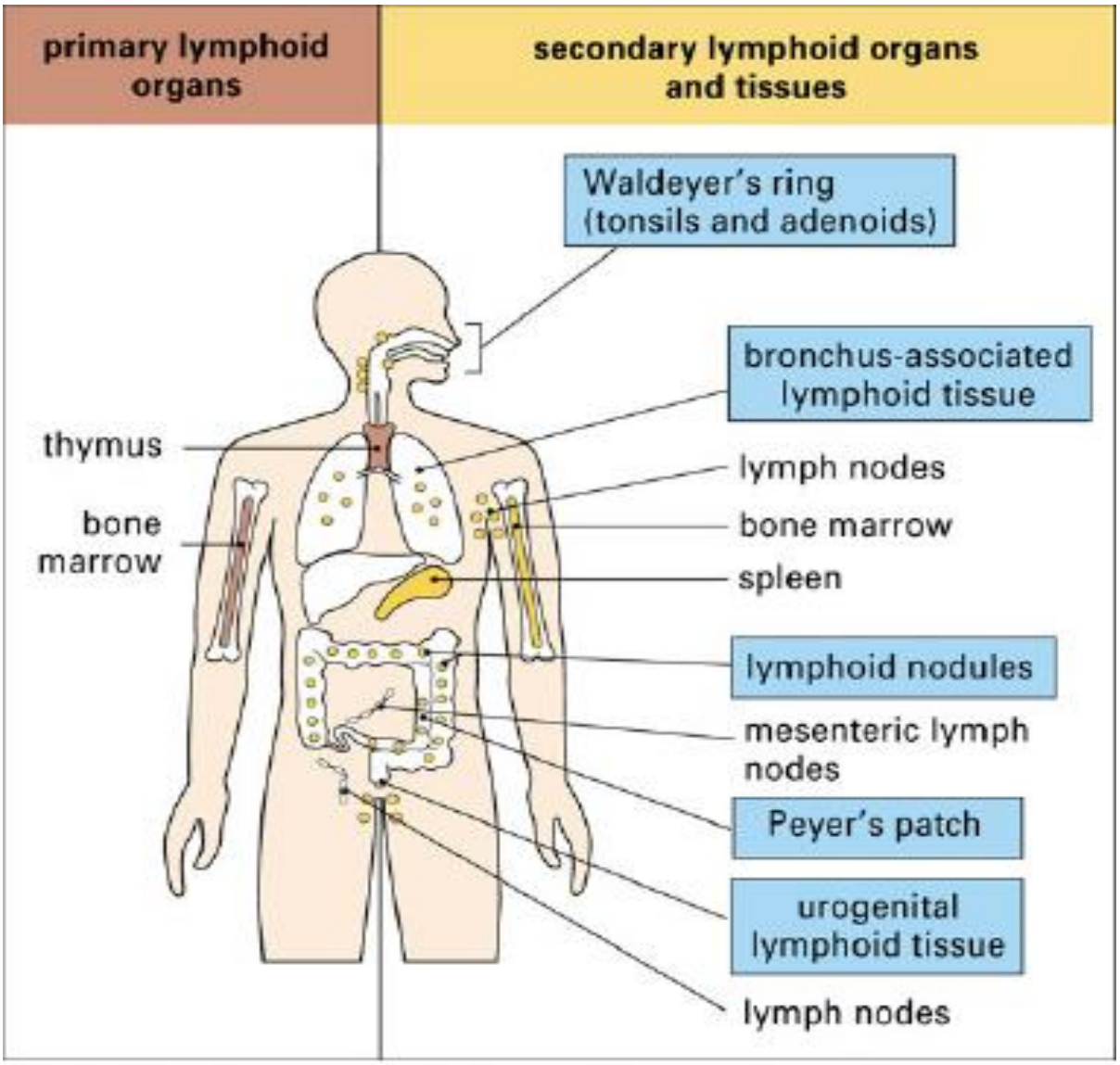

primary lymphoid organs
secondary lymphoid organs and tissues
Waldeyer's ring (tonsils and adenoids)
bronchus-associated lymphoid tissue
thymus
lymph nodes
bone marrow
bone marrow
spleen
lymphoid nodules
mesenteric lymph nodes
Peyer's patch
urogenital lymphoid tissue
lymph nodes

Figure 1-1: Organs of the immune system. The organs of the human lymphoid system are classified into two categories: primary and secondary. The primary organs are the bone marrow and the thymus, whereas the secondary lymphoid organs include spleen, lymph node, tonsils, and Peyer's patches. The figure was obtained with permission from Elsevier publisher. (Permission from the publishing group is depicted in the appendix) (195).

1.1.1 The Innate Immune System

The innate immune system represents the first line of defense against common invasions. It includes the physical and chemical properties of the skin, as well as mucosal surfaces of respiratory, urinary, and gastrointestinal tracts to prevent microorganisms from entering the body. Epithelial cells lining those mucus membranes utilize a variety of defense mechanisms to defeat the pathogens. For instance, mucus secretion, acidity and digestive enzymes of the gastrointestinal tracts, and several antimicrobial peptides produced by epithelial cells at the site of infection (e.g., lysozyme and β-defensins), all play a pivotal role in limiting the propagation of infection (4, 5). If a pathogen manages to overcome this first line of epithelial barriers, it encounters cells of the innate immune system that will recognize, engulf, and destroy the invading pathogens.

Most of the cells of the innate immune system are myeloid in origin and include circulating cells (neutrophils, eosinophils, and basophils) and tissue-resident cells, such as macrophages and dendritic cells (DCs). Other innate effector cells are of lymphoid lineage and include natural killer cells (NK), natural killer T cells (NKT), and γδ T cells. Upon invasion of microorganisms, these cells are rapidly recruited to the site of infection, thus providing an immediate protective response. These cells utilize an array of germ-line encoded receptors, known as pattern-recognition receptors (PRRs), to eliminate the pathogen. These PRRs are either found on the surface of cell membrane or within intracellular compartments. These receptors recognize constitutive and conserved microbial structures, such as components of the bacterial cell wall or viral nucleic acids, which are referred to as pathogen-associated molecular patterns (PAMPs). In addition to PAMPs, PRRs have been reported to recognize danger signals from damaged cells known as damage-associated molecular patterns (DAMPs) (6).

PRRs are grouped into different classes, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), complement receptors, C-type lectin receptors (CLRs), and scavenger receptors (7). Among PRRs, TLRs have been well characterized and extensively studied. There are approximately 10 functional TLRs in human cells, and most of their respective ligands have been identified (8). TLRs 1, 2, 4, 5, and 6 are expressed on the cell membrane, where they recognize mostly bacterial infection. For instance, TLR2 recognizes peptidoglycan from Gram-positive bacteria and bacterial lipoproteins (9). TLR4 was the first mammalian TLR to be discovered that binds to lipopolysaccharide (10). The bacterial flagellin that is essential to form bacterial flagella is recognized by TLR5 (11). In contrast, TLRs 3, 7, 8, and 9 are expressed in the membranes of endosomes and lysosomes, where they detect mostly microbial nucleic acids. Specific examples include the ability of TLR3 and TLR9 to recognize double-stranded ribonucleic acid (RNA) viruses and unmethylated CpG in bacterial deoxyribonucleic acid (DNA), respectively (12, 13). Upon ligation with their respective ligands, activated TLRs initiate intracellular signaling cascades. This initiation activates the transcription of genes encoding inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interferon genes (IFNs), chemokines, and antimicrobial compounds (14).

In addition to the cellular aspects of innate immunity, a response mediated by complement proteins plays a central role in the innate defense. There are three common pathways through which the complement system can be activated: the classical pathway, the alternative pathway, and the mannan-binding lectin pathway. The classical pathway is triggered by complement binding to the antigen-antibody complex, while the alternative and mannan-binding lectin pathways are initiated by complement binding directly to the surface of the pathogen (15). Upon complement activation, several biochemical cascade reactions

occur where activated complement proteins act as proteolytic enzymes. This recruitment of complement proteins eventually triggers the formation of the so-called “membrane attack complex”, which causes holes in the pathogen membrane, thus inducing the degradation of pathogens (16).

Another important effector function of the complement system is to coat microbes with complement proteins—a process called opsonization—in order to activate phagocytosis (15). Phagocytosis is considered one of the most important effector functions of the innate immune cells. Virtually all cells participating in innate immunity are effective phagocytes. Upon recognition by phagocytes, pathogens are engulfed and trapped into a type of vesicle called a phagosome. The acidity of this vesicle is gradually increased, ultimately fusing with the lysosome and forming phagolysosomes. Digestive enzymes and reactive oxygen species (ROS) within the phagolysosomes eventually target the microbe for destruction (17). The defense mechanisms of innate immunity might be sufficient to clear invasions, but if not, they can limit infection until cells of the adaptive immune response have been activated and expanded enough to ensure elimination of the threats.

1.1.2 The Adaptive Immune System

After a non-specific response from the innate immune system, adaptive immunity is required to provide a highly specific response for protection against a pathogen. This protection response develops immunological memory, a distinctive feature of the adaptive immune system. Cells of the adaptive immune system are able to distinguish between proteins produced by the normal cells (self-antigen) from proteins produced by altered or infected cells (non-self-antigen) (18). The response of the adaptive branch of immunity can be categorized

into humoral and cellular immune responses, which are mediated by B lymphocytes (B cells) and T cells, respectively.

In contrast to the innate responses that use limited receptors to recognize conserved microbial structures, cells of the adaptive immune system express receptors that specifically detect and adapt to a large variety of antigens of different nature, thereby complementing the function of the innate immunity. This is due to somatic gene rearrangements, also known as the variable (diversity) joining (V(D)J) recombination process, which generates a diverse repertoire of B cell receptors (BCRs) and T cell receptors (TCRs) capable of recognizing countless numbers of foreign invaders (19). The pathogenic molecules can be directly recognized by BCRs on the surface of B cells that further drive their activation. In order to activate T cells, antigens must first be processed and presented by major histocompatibility complex (MHC) molecules on antigen presenting cells (APCs). More details on antigen presentation and APCs are described in Section1.2.2

Following B or T cell activation, the cells undergo rapid clonal expansion through a process of mitotic cell division. This process results in the generation of a large number of effector cells. The resulting daughter cells, in the case of B cells, are called plasma cells. These cells are the source of antibody production. Upon secretion, the specific antibodies can take two roles: i) neutralizing antibodies, which hinder the adhesion of pathogens to their target host cells; or ii) opsonizing antibodies, which mark the microbes for subsequent elimination through phagocytosis. Regarding opsonizing antibodies, this elimination is either performed by a process known as antibody-dependent cellular cytotoxicity (ADCC), or by complement activation (20).

Activated T cells, on the other hand, differentiate into two distinct phenotypes of effector cells depending on the type of peptides presented by APCs. Cytotoxic T cells ($CD8^+$

T cells) recognize antigens presented by MHC class I molecules (MHC I), and these cells are then activated to differentiate into effector $CD8^+$ T cells. Helper T cells ($CD4^+$ T cells) recognize antigens presented by MHC class II molecules (MHC II), and these cells then differentiate into effector $CD4^+$ T cells. Successful activation of T cells in turn promotes the killing of infected cells via the release of cytotoxic molecules, such as perforin and granzymes by $CD8^+$ T cells. It also results in the secretion of inflammatory cytokines by $CD4^+$ T cells, which then activate the proliferation and differentiation of other cells of the immune system (21, 22). More details on T cell activation and function are described in the following sections.

Once the pathogen has been cleared, a critical negative regulatory mechanism takes place in order to avoid over-activation of lymphocytes, which could lead to immunopathology. During this contraction phase, the vast majority of activated antigen-specific T cells undergo programmed cell death; however, a small proportion turn into memory T cells (23). The development of immunological memory is an important aspect of adaptive immunity, in which subsequent exposures to a specific pathogen will elicit a more rapid and stronger response than the primary response.

1.2 T Lymphocytes

1.2.1 Development and Maturation

T cell development originates in the BM or in the liver during embryonic life. Hematopoietic stem cells (HSCs) differentiate into common lymphoid progenitors that in turn enter the bloodstream and migrate to the thymus, where the maturation process takes place (24). During thymopoiesis, a series of maturation stages occur that generate mature T cells. These T cells possess functional TCR (either TCRαβ or TCRγδ) and express either CD4, or

CD8 in the case of αβ T cells on their cell surface. Once the cells have migrated to the thymus, they circulate through different anatomical zones in the thymus where they receive signals required for complete T cell maturation (25).

The first phase of the development is the double negative (DN) stage, in which thymocytes do not express CD4 and CD8 on their surface. The cells at this stage can be subdivided into four distinct phenotypes based on the expression of CD25 (interleukin-2Rα) and CD44 (phagocyte glycoprotein 1): DN1 ($CD25^{-}CD44^{+}$), DN2 ($CD25^{+}CD44^{+}$), DN3 ($CD25^{+}CD44^{-}$), and DN4 ($CD25^{-}CD44^{-}$) (26). The rearrangements of TCR β, γ, and δ genes are initiated at the DN2 stage and successfully are formed at the DN3 stage (27). The cells at the DN3 stage possess incomplete αβ TCR complex, which has a full chain of the TCRβ subunit and pre-α chain (28). Signals through the pre-TCR complex subsequently rescue the cells from dying, allowing the formation of functional TCRαβ and progression to the double positive (DP) stage. The maturation process is, however, not considered complete until the DP thymocytes are functionally screened for the recognition of self-antigens presented on MHC molecules in a process called positive selection. Therefore, low affinity binding to the peptides presented by MHC I or MHC II will result in maturation of $CD8^{+}$ or $CD4^{+}$ T cells, respectively. Once the maturation is completed, mature T cells exit the thymus, and αβ T cell subsets migrate to the SLOs where they reside, while γδ T cell subsets mostly reside in the epithelial and mucosal tissues (29). Thymocytes that express highly reactive TCR or defective receptors are eliminated via apoptosis (30).

1.2.2 Antigen Presentation and T cell Activation

In the periphery, naïve T cells (T_N) continuously circulate through the bloodstream and SLOs in search of antigens (31). Activation of T cells requires the antigens to be processed and presented first by APCs expressing MHCs to T_N. There are two classes of MHCs: MHC I, expressed by non-professional APCs (all nucleated cells), and MHC II, expressed by professional APCs (DCs, macrophages, and B cells).

Typically, MHC I present antigens derived from intracellular protein of viruses or bacteria. These antigens are first degraded in the cytosol of an APC by nuclear proteases. The resulting peptides travel to the endoplasmic reticulum (ER) via the transporter associated with antigen presentation, and are then loaded onto an MHC I and assembled in the ER. Once a stable peptide-MHC I complex is generated, the complex leaves the ER via the Golgi apparatus and then translocates to the APC surface for recognition by the TCR of $CD8^+$ T cells (32).

On the contrary, exogenous antigens, such as bacteria, toxins, or parasites, are phagocytosed by professional APCs and degraded into small peptides displayed by MHC II (33). Unlike MHC I, MHC II in the ER is occupied by a chaperone protein called the invariant chain. The invariant chain prevents peptides from binding to MHC II in the ER (34). The loading of peptides onto MHC II takes place in the endosomal vesicles. The acidic nature of the endosomal proteases mediates the cleavage of the invariant chain, thus allowing the peptides to replace the degraded chain and integrate into the peptide-binding site of MHC II (35). Finally, the generated peptide-MHC II complex is transported to the cell membrane, where the antigen is then displayed to $CD4^+$ T cells.

The engagement of the TCR with cognate MHC-peptide complexes represents the first signal for T cell activation. To be fully activated, T cells must also receive a subsequent stimulatory signal through the interactions between other co-stimulatory molecules. This

includes: i) the interaction of CD28 on T cells with its ligands CD80 or CD86, expressed on the surface of APCs; and ii) the binding of intercellular adhesion molecule-1 (ICAM-1) on APCs to integrin leukocyte function-associated antigen-1 (LFA-1) on T cells (36). These signals aid in stabilizing the connection between T cells and APCs, and enhance the T cell responses. Altogether, the activation of T cells leads to the release of specific cytokines, the upregulation of cytokine receptors, the clonal expansion of antigen-specific T lymphocytes, and the differentiation of naïve T cells into effector and memory T cells.

1.2.3 Effector T cell Subsets and Memory Cell Formation

Upon activation, T cells start to produce interleukin 2 (IL-2) and upregulate IL-2 receptors, leading to the rapid proliferation and clonal expansion of the activated T cells (37). Based on the initiated stimuli and the cytokines secreted by APCs and surrounding cells, T_N cells differentiate into a distinct subset of effector cells that have a specific immunological function.

1.2.3.1 $CD4^+$ T cells

$CD4^+$ T_N cells have been reported to differentiate into a vast array of effector T helper (Th) subsets as will be discussed below. These include but are not limited to Th1, Th2, Th17, and T regulatory (Treg) cells (38).

The Th1 cells play important roles in the eradication of intracellular pathogens (bacteria, viruses, and some protozoa). This is mediated by the secretion of the signature cytokines IFNγ, IL-2 and TNFα, which promote the antimicrobial activity of the macrophage and the cytotoxic effect of T and NK cells (39). Commitment to the Th1 lineage is promoted by the cytokine IL-12, the transcription factors, signal transducer and activator of transcription proteins 4 (STAT4), and the master regulator of Th1 differentiation T-box transcription factor

(T-bet) (40–42). In the case of Th2 cells, activated T cells enhance the clearance of parasitic infections through the production of IL-4, IL-5 and IL-13. These cytokines recruit basophils, eosinophils, and mast cells to the site of infection. In addition, these cytokines promote the production of IgE antibody from B cells, a potent antibody against parasitic infections (43). Differentiation of Th2 lineage cells is promoted by IL-4, the transcription factors STAT6 as well as the master regulator of Th2 differentiation, GATA3 (43, 44).

Th17 cells are another subset of $CD4^+$ T cells, distinct from Th1 and Th2 subsets (45). These cells produce IL-17A and IL-17F upon activation, as well as IL-21 and IL-22. Differentiation of cells toward a Th17 cell lineage requires signals by IL-1β, IL-6, IL-21, IL-23, and TGF-β (46–49). Th17 cells appear to play a protective role in the eradication of the extracellular bacterial and fungal pathogens, by mediating the recruitment of neutrophils and macrophages to the site of infection (50, 51). However, it has been reported that Th17 may also contribute to the pathogenesis of some inflammatory and autoimmune disorders (52, 53). Differentiation of cells into the Th17 lineage is promoted by the following: interleukins IL-6 and IL-23, the transcription factors STAT3, and the master regulator of Th17 differentiation retinoic acid receptor-related orphan receptor-γt (ROR-γt) (39).

In addition to the aforementioned $CD4^+$ subsets, there are regulatory $CD4^+$ T cells (Treg cells), which play an important role in the suppression of immune responses following the activation of the immune cells. The suppressive effects are mediated either through direct cell to cell contact or through the secretion of anti-inflammatory cytokines, such as IL-10 or TGF-β (54). Some of the Treg cells naturally arise from the thymus, while others are generated after the activation of T cells and are called inducible Treg cells. FOXP3 is the master transcription factor for Treg cells; it plays an essential role in the development and maintenance of Treg cells (55).

1.2.3.2 $CD8^+$ T cells

The activation of $CD8^+$ T_N cells leads to their rapid proliferation and clonal expansion, which in turn results in their differentiation into effector cytotoxic T cells (CTLs). The destruction of target cells by CTLs is governed through the release of cytotoxins stored within specialized lytic granules into the surface of target cells. These cytotoxins include granulysin, which has antimicrobial activity and induces the apoptosis of target cells, as well as perforin, which helps form pores in the plasma membrane of the target cell (56, 57). Next, the CTLs release granzymes that enter target cells via the perforin-formed pore and induce DNA damage. The induced damage initiates apoptosis through both caspase-dependent and caspase-independent pathways (58). Moreover, activated CTLs upregulate the expression of FasL, which will bind to the Fas receptor on target cells and initiate the apoptotic machinery in infected cells (59).

1.2.3.3 Memory T cell Formation

Following the successful clearance of a pathogen by the immune response, many of the effector T cells (T_E) (>90%) undergo apoptosis or programmed cell death (60). However, some of the effector cells persist to become memory T cells (T_M). These T_M are quiescent and long-lived. The main feature of the T_M is that they are capable of responding rapidly upon encountering the pathogen in secondary infection. To maintain their sustained growth, T_M mainly rely on the cytokine signals delivered by interleukin 7 (IL-7) and interleukin 15 (IL-15). The receptor for these cytokines is thus highly expressed on the surface of memory cells (61, 62). The mechanisms governing the generation of T_M remain controversial.

Some reports indicate that T_M are generated by differentiating T_N into T_M via effector cells (63, 64). Other studies suggest that T_M might be generated without going through the intermediate effector stage of differentiation (65). A consistent finding between these models is that the T_M can be grouped into two distinct populations of $CD4^+$ and $CD8^+$ T_M cells, central memory T cells (T_{cm}) and effector memory T cells (T_{eff}). The different T_M phenotypes are defined by the presence or absence of three cell surface markers: the activation marker CD44, the cell adhesion molecule L-selectin (CD62L), and the C-C chemokine receptor type 7 (CCR7) (66). T_{cm} have been found to express ($CCR7^+$ $CD62L^+$ $CD44^+$) and reside in the blood and lymphatic tissues, while T_{eff} lose the expression of both markers ($CCR7^-$ $CD62L^-$) and express $CD44^+$,and home to peripheral tissues (66). Although these two subsets of T_M exist, the factors mediating their generation are not well understood.

Additionally, T_M cells can be generated in the absence of foreign antigen. Following T cell lymphopenia, T_N can undergo homeostatic proliferation and can differentiate into T_M cells in response to self-antigens and IL-7 signals (67–69). These T_M cells are referred to as memory-like or memory phenotype cells. Although these cells have not been generated by contact with a foreign antigen, they retain functional and phenotypic characteristics of conventional T_M (70–72). These memory-like T cells result from differentiating T_N in response to self-antigens following lymphopenic stress. In some instances, they are capable of reverting to their naïve phenotype once the T cell compartment is restored to its normal homeostatic level (71).

1.3 Cytokines, Interleukins, and Interleukin 7

Cytokine is a broad term for small, secreted proteins/peptides that are often involved in regulating immune responses. This includes cell development, differentiation, signaling, and activity. There are several different families of cytokines that include chemokines, interferons, and interleukins (73). Interleukins were first thought to be small signaling molecules produced by leukocytes to target other leukocytes, but they now have a broader definition with more target cells. Interleukins are a type of cytokine, but not all cytokines are interleukins. Approximately 40 interleukins have been identified so far, and have been subdivided into several families based on their receptor similarities and functional properties (74–76). One of the important interleukin families is the common γ chain cytokines, which consist of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (77). This thesis will focus on IL-7, such as its biology and site of production (Section 1.3.1); the signaling and regulation of the interleukin 7 receptor (Section 1.3.2); and the pleiotropic effects of IL-7 on T cells (Section 1.3.3) will be discussed.

1.3.1 Biology of IL-7 and Its Site of Production

IL-7 is a hematopoietic growth factor and a master regulator of T cell development and homeostasis. IL-7 is a single-chain glycoprotein (25 kDa), encoded by a gene located on chromosome 3 in mice and chromosome 8 in humans (78, 79). It was originally discovered as a cytokine that promotes the growth of murine B cell precursors, and then was identified as an essential cytokine for murine and human T cell homeostasis (79, 80). Although not produced by T cells, a variety of other cell types produce IL-7 including: stromal cells in the thymus and BM, vascular endothelial cells, intestinal epithelial cells, keratinocytes, and follicular DCs

(81). In healthy individuals, the plasma level of IL-7 has been found to be <10 pg/ml (82), and the production of IL-7 is believed to occur at a constant rate. Hence, it is suggested that the regulation of IL-7 concentration is mediated by lymphocyte consumption and competition for the limiting amount of IL-7, rather than by its production (81).

1.3.2 Interleukin 7 Receptor: Signaling and Regulation

IL-7 signals are transduced by the IL-7 receptor (IL-7R). The receptor is a heterodimer complex, composed of an IL-7R-alpha chain (CD127) and the common gamma chain (CD132/IL-2Rγ/γc). The IL-7 Rα gene is located on chromosome 15 in mice and chromosome 5 in humans (78, 83). The gene consists of 8 exons, which encode for a protein of 459 amino acids (aa). The full length of CD127 protein consists of 20 aa for signal sequence, 219 aa for extracellular domain (encoded by the first 5 exons), 25 aa that make the transmembrane domain (encoded by exon 6), and 195 aa that form the cytoplasmic tail (encoded by exons 7 and 8) (84–86). CD127 is also a part of the thymic stromal lymphopoietin receptor. CD132 is shared by other common γ chain cytokines, including IL-2, 4, 9, 15 and 21 (87–89).

Binding of IL-7 to its receptor triggers the dimerization of the two receptor chains, allowing the activation of the tyrosine Janus kinases (JAKs), JAK 1 and JAK 3 which are associated to the cytoplasmic tail of CD127 and CD132, respectively (86, 90, 91). This complex promotes downstream phosphorylation processes, and initiates a number of signaling cascades. Two of the major signaling pathways activated by IL-7 are JAK/STAT, and phosphatidylinositol 3 kinase /protein kinase B (PI3K/AKT) (78).

For JAK/STAT signaling, IL-7 ligation induces phosphorylation of JAK 3 and JAK 1, as well as the tyrosine residues in the cytoplasmic part of CD127. This phosphorylation creates

a docking site for the SH2 domain, which leads to the recruitment of STAT 1, 3, or 5 (92, 93). STAT 5 is considered the main STAT protein activated by IL-7, and two isoforms have been reported for STAT 5, STAT5a and STAT5b (86). The tyrosine Y449 site is crucial for the downstream signal of IL-7. Once phosphorylated, it recruits STAT 5 where it is subsequently phosphorylated by JAK and other relevant kinases (78). The phosphorylated STATs subsequently dimerize and translocate to the nucleus, where they regulate the activation of cytokine dependent genes involved in cell survival and proliferation of T cells (94, 95).

IL-7 also induces the activation of PI3K (96). This is mediated by the phosphorylation of PI3K p85 subunit and is activated by JAK 3 (97). AKT is a serine/threonine kinase and is a major target of PI3K signaling, which in turn is responsible for regulating a number of important factors essential for cell survival (86, 98). Other effector functions of PI3K/AKT signaling pathways have been attributed to the activation of cell proliferation and the maintenance of glucose metabolism (99). Signaling of IL-7 has a central role throughout the lifespan of T cells; it is required for both thymopoiesis and T cell maturation (100). After maturation, IL-7 signaling promotes cell survival and maintains homeostasis of T_N and T_M cells via proliferation (101, 102). In addition, the cytolytic function of CTLs, in response to foreign antigens, is enhanced by IL-7 (103, 104).

IL-7R is expressed on cells of lymphoid origin, such as developing and mature T cells, and non-immune cells such as stromal cells of the bone marrow (100, 105). Loss of the receptor due to a genetic defect, or manipulation in mice, leads to severe combined immunodeficiency (SCID) with a complete block in T cell development (106). Aberrations in CD127 expression or dysregulation of IL-7 levels are associated with several diseases, including HIV, autoimmune diseases, and different types of cancer (107–110). Given the importance of IL-7

in T cell homeostasis, the mechanisms modulating IL-7 signaling through its cognate receptor are tightly regulated, but the underlying processes of that regulation are not well characterized.

Research has shown that intrinsic feedback controls are important in the regulation of CD127 expression. Interestingly, several studies have demonstrated that receptor ligation with IL-7 decreases the expression of surface CD127 at both the transcriptional and translational level (111, 112). In addition to the membrane receptor form of CD127, a soluble form (sCD127) has been identified. This soluble receptor has the capability to bind with IL-7. As such, one can hypothesize its involvement in the regulation process (88).

1.4 Pleiotropic Effects of IL-7 on T cells

1.4.1 IL-7 and Thymopoiesis

The essential roles of IL-7 in the development of T cells are unique and indispensable during the entire process of thymopoiesis. This is evident in the murine study findings that IL-7/IL-7Rα deficiency, or treating mice with IL-7/IL-7Rα neutralizing antibodies, result in severe lymphopenia and inhibition of T cell development (80, 106, 113, 114). Moreover, mutations of the CD127 gene in humans result in severe combined immunodeficiency (SCID), characterized by abnormal development of T cells while maintaining normal lymphopoiesis of B and NK cells (115). The above findings confirm the crucial role of IL-7 in human T cell thymopoiesis, and conclude that the defect on T cell development is more pronounced in CD127 deficiency than in IL-7 deficiency. This is due to the additional loss of Thymic Stromal Lymphopoietin signaling, which if present can lessen the effects of IL-7 deficiency (116). In the thymus, IL-7Rα has been found to be highly expressed in DN thymocytes, silenced and

down-regulated on DP subsets, and re-expressed upon completing the maturation of T cells on single positive T cells ($CD4^+$ or $CD8^+$ T cells) (117) **Figure 1-2**.

The effects of IL-7 on T cell thymopoiesis are characterized by multiple stages of the development of T cells, which include the differentiation of DN thymocytes and the rearrangement of TCR (118). In the DN stage, CD127 expression is essential for the survival of DN subsets by preventing programmed cell death, due to the upregulation of the anti-apoptotic molecule BCL-2 (119). It has been shown in IL-7Rα $^{-/-}$ mice that overexpression of BCL-2 genes or deletion of the pro-apoptotic molecule BCL-2 associated X protein (BAX) can partially rescue T cell development (120–122). Further, IL-7 signaling is critical for DN subset differentiation and proliferation. This is mediated by activating PI3K downstream signals, which, in addition to stimulating cell cycle progressions, is required to overcome a T cell development inhibitory signal rendered by phosphatase and tensin homologue deleted on chromosome 10 (123, 124). IL-7 has also been shown to play an essential role for the γ-chain rearrangement of TCR, and also contributes to the β TCR rearrangement by increasing histone acetylation and chromatin accessibility (125, 126). Interestingly, the absence of IL-7 signals can result in a complete lack of γδ T cell population and a dramatic loss of the αβ T cells (118).

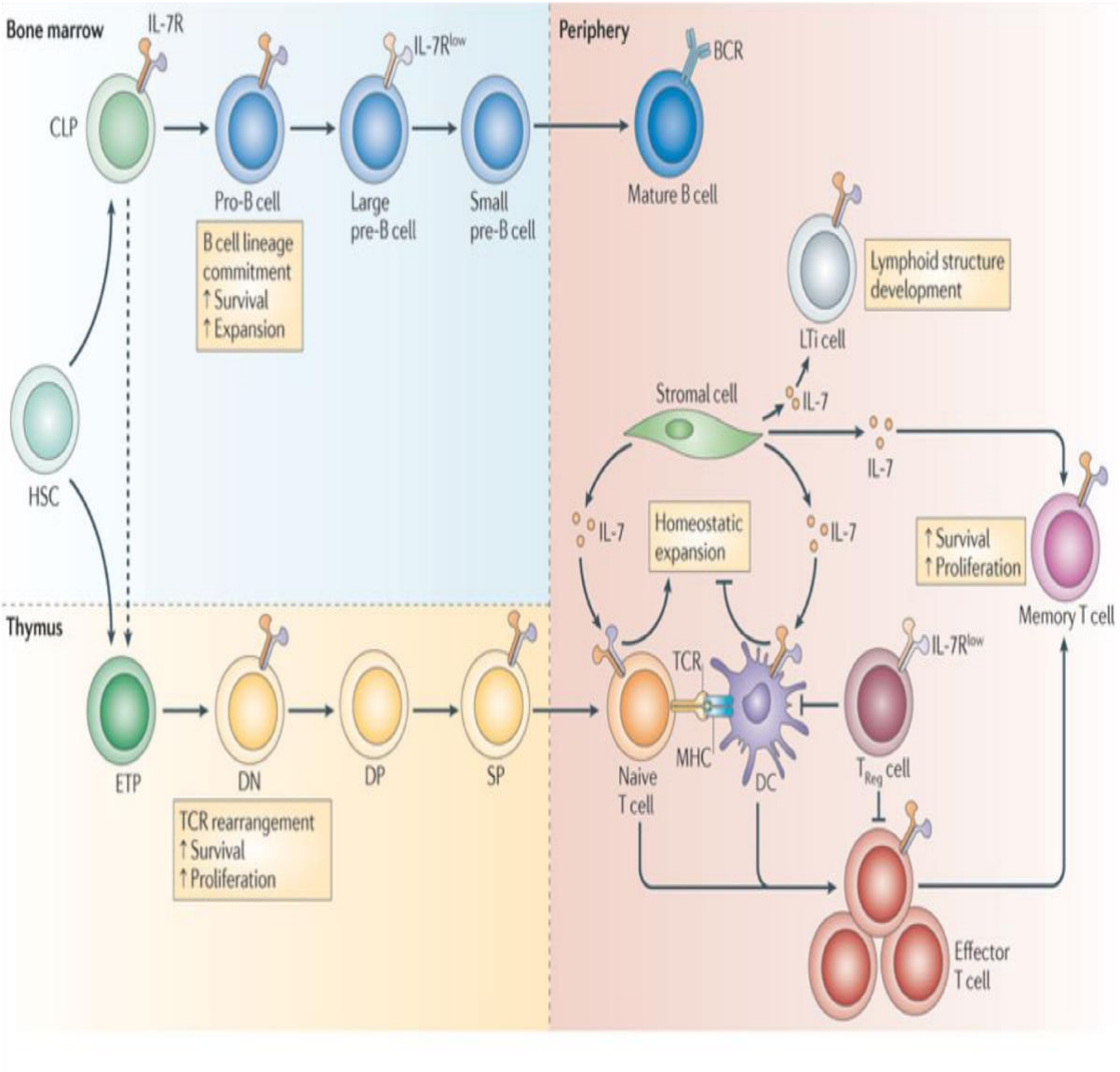

Bone marrow
IL-7R
CLP
Pro-B cell
IL-7R^{low}
Large pre-B cell
Small pre-B cell
B cell lineage commitment
↑ Survival
↑ Expansion
HSC
Thymus
ETP
DN
DP
SP
TCR rearrangement
↑ Survival
↑ Proliferation
Periphery
BCR
Mature B cell
Lymphoid structure development
LTi cell
Stromal cell
IL-7
IL-7
IL-7
IL-7
Homeostatic expansion
↑ Survival
↑ Proliferation
Memory T cell
TCR
MHC
Naive T cell
DC
IL-7R^{low}
T_{Reg} cell
Effector T cell

Figure 1-2:The requirement of IL-7 signaling throughout the life-span of lymphocyte. During B cell development, IL-7 signaling promotes commitment of progenitors to B cell fate in the bone marrow. The expression of IL-7Rα is then downregulated on mature B cell population. During T cell development, IL-7 signaling promotes the survival and expansion of DN thymocytes. The expression of IL-7Rα is then downregulated on DP thymocytes and re-expressed by SP thymocytes. In the periphery, mature naive T cells express IL-7Rα. Upon encountering foreign antigens and subsequent activation, naive T cells differentiate into effector T cells and lose the receptor. Upon clearance of the pathogen, a small number of effector T cells retain the expression of IL-7 receptor and constitute the pool of long-lived memory cells. The figure was obtained with permission from Springer Nature. (Permission from the publishing group is depicted in the appendix) (127).

1.4.2 IL-7 and Mature T cell Homeostasis and Proliferation

In addition to the requirement of IL-7 on T cell development, IL-7 plays a vital role in T cell homeostasis by maintaining a constant number of T cells in the periphery, and by controlling cell death and division. The survival of mature T cells is dependent on IL-7 signaling in addition to the weak interaction with self-peptide/MHC ligands via the TCR (128, 129). Previous findings have shown the necessity of IL-7 for T_N survival. A report published by Tan et al., 2001 demonstrated that transferring naïve $CD4^+$ and $CD8^+$ T cells into IL-7 deficient mice abrogated the recovery of the cells and observed that one month following the adoptive transfer the cells had completely disappeared (130). Further, Vivien et al., 2001 revealed that T_N number was dramatically reduced via blocking IL-7 signaling by administering anti-IL-7R antibodies in mice (131).

IL-7 prompts cell survival through multiple mechanisms. These include the inhibition of apoptosis by upregulating anti-apoptotic molecules B cell lymphoma 2 (BCL-2) and myeloid leukemia cell differentiation protein 1 (MCL-1), and the inhibition of the pro-apoptotic molecules BAX, BAD, and BIM (119, 132–134). Additionally, IL-7 has also shown to promote T cell survival through stimulating glucose uptake and maintaining cellular metabolism. Lum et al., 2005 showed that in cell lines lacking essential proteins for apoptosis, progressive atrophy occurred due to reduced glycolytic activities, reduced ATP production, and decreased glucose transporter GLUT1 (135). Hence, the finding that IL-7 induces GLUT1 expression and enhances glucose uptake highlights the potential link between IL-7 and T cell survival (136).

In addition to the requirement of IL-7 in T cell survival, IL-7 stimulates the proliferation of T cells by regulating factors important for cell division (101). These factors include different classes of cyclin-dependent kinases (cdks) essential for cell-cycle progression

(e.g., cdk2 and cdc25A) as well as negative regulators of cell proliferation known as cyclin-dependent kinase inhibitors (CKIs) such as $p27^{kip1}$ (137). IL-7 has been reported to primarily regulate cell proliferation by inducing the upregulation of cdc25A and downregulation of cell cycle inhibitor, $p27^{kip1}$ (137, 138). Under normal conditions, when T cell numbers are not altered, there is relatively minimal need to activate homeostatic cell proliferation. Thus, the IL-7 signals mainly promote cell survival and maintenance of T cell homeostasis. Whereas in lymphopenic conditions, IL-7 can stimulate T cell proliferation in order to restore T cell numbers (118).

Several studies have elucidated the enhanced effect of IL-7 on T cell proliferation in pathological conditions that cause a reduction in T cell number. In one study, a 3-week treatment of SIV-infected non-human primates with IL-7 showed a significant increase in the number of peripheral T cells (139). Similarly, in HIV-infected individuals receiving antiretroviral therapies, an enhanced recovery of $CD4^+$ and $CD8^+$ T cells was observed by administering IL-7 therapy (140).

1.5 Soluble Cytokine Receptors

The first soluble cytokine receptor identified by Rubin et al. was the soluble IL-2 receptor in 1985 (141). Since this initial discovery, other studies suggest that most cytokine receptor families have soluble cytokine receptors. Generally, soluble cytokine receptors arise from the extracellular protein of the membrane-bound receptor which retain the ability to bind their ligands. There are several mechanisms through which a soluble receptor can be generated. It can result from a proteolytic cleavage of ectodomains mediated by a large family of metalloproteases, known as sheddase proteins, such as ADAMs (a disintegrin and

metalloproteinases) and MMPs (Matrix metalloproteinases) (142). Another mechanism involves alternative mRNA splicing, where the transmembrane domain(s) of the receptor is spliced out. Thus, the secreted receptor protein is in a soluble form (142).

The importance of studying the role of soluble cytokine receptors comes from the idea that these receptors can affect the balance between ligands and their relative cell membrane receptors, and thus alter immune responses. Generally, soluble receptors can either attenuate or promote cytokine signaling. In one model, soluble cytokine receptors exert antagonistic effects by acting as natural competitors of the membrane receptor (143). The higher the affinity of the soluble receptor for the ligand, the more potent the inhibition of the signal will be. A previous study of soluble IL-2Rα revealed that binding to the ligand reduces its ability to signal, inhibiting IL-2 dependent T cell activation and proliferation (144). Another report published by Arend et al., 1994 showed that soluble IL-1RII can attenuate IL-1α and β bioactivities by acting as a decoy receptor (145).

Contrary to the negative impact on cytokine signaling, soluble cytokine receptors have also been found to exert agonistic effects. Previous findings have demonstrated that soluble IL-4 and IL-15 receptors enhance the activity of their ligands by increasing cytokine stability and protecting them from degradation (146, 147). In some cases, soluble receptors bind to the ligands and trans-present them to cells that do not express the complete receptor, such as sIL-15Rα and sIL-6R (147, 148).

1.5.1 sCD127: Mechanism of Generation and Biological Effects on IL-7 Signaling

In 1990, the molecular cloning of human and murine cDNA encoding membrane-bound IL-7Rα by Goodwin et al. surprisingly revealed a soluble variant of the receptor (88).

The generation of sCD127 is primarily derived by alternative splicing of mRNA, when exon 6 (which codes for the transmembrane region of the membrane receptor (Δ6, sIL-7Rα)) is removed. This removal results in the generation of a unique 26-aa sequence, followed by a premature stop codon (149). In addition to the alternative splicing, sCD127 may be generated by ectodomain shedding. This process causes a proteolytic removal of the transmembrane domain of the membrane receptor via a shedding protease, resulting in the release of the soluble extracellular domain of the receptor. Unpublished work from our lab has hypothesized that MMP 9 may have a role in the generation of sCD127 through proteolytic shedding; however, the full mechanism of sCD127 generation by this mechanism remains to be confirmed.

Despite the fact that soluble receptors are known to mediate cytokine signaling, the exact biological effects of sCD127 *in vivo* have not been well-elucidated. The importance of delineating the role of sCD127 in mediating IL-7 activities arises from the fact that in healthy individuals, sCD127 has been found to circulate at high concentrations compared to IL-7, nmol/ml compared to pmol/ml levels, respectively (85). Also, various degrees of affinity to bind IL-7 have been reported. For instance, moderate-affinity to IL-7 has been found when sCD127 is in homodimer form, whereas the heterodimer form sCD127/sγc has a high binding affinity to IL-7 (150). Both attenuating and promoting effects on IL-7 bioactivity have been described. In some instances, sCD127 was reported to block IL-7 signaling as well as inhibit STAT5 phosphorylation and IL-7-mediated proliferation (151, 152). However, other studies have demonstrated that sCD127 acts as a carrier protein for IL-7, thereby increasing the half-life of IL-7 and promoting the biological activity of IL-7 (150).

Furthermore, a recent study in our lab has reported that pre-incubation of a chimeric IL-7Rα-Fc receptor (an artificial protein that mimics natural sCD127, consisting of CD127

extracellular domain and human FC-fragment) and IL-7 enhanced IL-7-mediated human and murine T cell proliferation *in vitro* (153). Such an observation paved the way for subsequent investigators to establish the role of IL-7 *in vivo*, which will be the main focus of my thesis.

1.5.2 Association Between sCD127 and Diseases

The presence of sCD127 in body fluids is readily demonstrated, and its relative abundance has been linked with various diseases, including autoimmune and infectious disorders. The findings demonstrate that a single nucleotide polymorphism (SNP) rs6897932 in IL-7Rα gene resulted in increased production of sCD127, which is linked to an increase in the risk of developing autoimmune diseases (e.g., multiple sclerosis [MS], lupus erythematosus, sarcoidosis, and type 1 diabetes [T1D]) (150, 154–156).

In the case of MS, the rs6897932 CC polymorphism is implicated in increasing the skipping of exon 6 and elevating the level of sCD127 production, which in turn enhanced the bioactivity of IL-7 by diminishing its consumption, and thus increased the susceptibility to MS (150). Similarly, an elevated level of sCD127 has been found in the serum of patients with systematic lupus erythematosus compared to healthy individuals. Upon treatment, the serum concentration of sCD127 significantly declined (154). Moreover, a previous report by Monti et al., 2013 revealed that serum concentrations of sCD127 were only increased in the onset of T1D within cohort of patients with positive islet autoantibody as compared with concentrations in age-matched first-degree relatives control group (152).

In addition to autoimmune diseases, sCD127 has been found to play a role in the context of infectious diseases. For instance, increased levels of sCD127 have been found in the plasma of HIV-infected individuals compared to healthy controls (151). As well, the SNP rs6897932 CC allele in CD127 gene has been associated with elevated levels of sCD127 in Caucasian HIV+ patients, which is associated with a slower rate of $CD4^+$ T cell recovery upon receiving combined antiretroviral therapy, and rapid AIDS progression (157, 158).

1.6 Hypothesis

IL-7 is critical for stimulating T cell proliferation, making it a potential therapeutic agent for treating clinical lymphopenic conditions. Furthermore, the *in vivo* functions of sCD127, an IL-7 receptor, are still unknown. I hypothesize that by administering exogenous sCD127, IL-7 activity should be enhanced, thus enhancing T cell proliferation *in vivo.*

Objectives:

1) To determine the effect of sCD127 treatment with or without exogenous IL-7 on T cell homeostasis in healthy mice.

2) To validate the use of CD4 and CD8 antibodies in order to generate a T cell-depleted mouse model, and determine the kinetics of their spontaneous T cell reconstitution.

3) To determine the effect of sCD127 treatment with or without exogenous IL-7 on T cell reconstitution and proliferation in the above T cell-depleted mouse model system.

2. Chapter 2: Materials and Methods

2.1 Mice

C57BL/6 mice (B6) were purchased from Charles River laboratories and kept under specific pathogen-free conditions in the main animal facility at the University of Ottawa. Mice used for experiments were between 7-12 weeks of age. Mice were age and gender-matched for all experiments. All experiments were performed according to ethical guidelines and were approved by the University of Ottawa Animal Ethics Committee and the Canadian Council on Animal Care.

2.2 Reagents

2.2.1 Antibodies

For T cell depletion, Rat anti-mouse anti-CD4 antibodies (Clone GK1.5 – catalog #: BE0003-1) and anti-CD8 antibodies (Clone YTS 169.4 – catalog #:BE0117) were purchased from Bio X Cell (Lebanon, USA). A complete list of the all antibodies utilized for surface and intracellular staining (flow cytometry) can be found in **Table 2-1**. Fixable Far Red Live/Dead was obtained from Invitrogen (Carlsbad, CA, USA).

Table 2-1. List of fluorochrome antibodies used for flow cytometry

Company	Target[a]	Reactivity[b]	Clone	Conjugate[c]	Catalogue#
BD Biosciences	Annexin-V	Ms	-	PE	51-65875X
	CD3	Ms	145-2C11	FITC	553062
	CD4	Ms	RM4-5	BV786	563727
	CD8	Ms	53-6.7	APC	553035
	CD19	Ms	ID3	APC/Cy7 and V450	557655, 560375
	CD44	Ms	IM7	PE	553134
	Ki-67	H, Ms, Rat, Rh	B56	V450 and BV421	561281, 562899
	NK-1.1	Ms	PK136	PE, PE/Cy7, and FITC	553165, 552878, 553164
	pSTAT5	H, Ms, Rat, Sh	pY694	PE	562077
	TCR-β	Ms	H57-597	FITC	553171
eBioscience	CD3	Ms	145-2C11	FITC	11-0031-85
	CD8	Ms	53-6.7	APC and PE/Cy7	17-0081-82, 25-0081-82
	CD62L	Ms	MEL-14	FITC	11-0621-85
	CD127	Ms	SB/199	PE	12-1273-81
	TCR-β	Ms	H57-597	APC and eFluor 450	17-5961-83, 48-5961-82
BioLegend	CD44 isotype	Rat	RTK4530	PE	400608
	CD62L isotype	Rat	RTK2758	FITC	400506
	CD127 isotype	Rat	RTK4530	PE	400608

[a]Target abbreviations: CD-Cluster of differentiation; CD62L-CD62 ligand; NK-1.1 – Natural killer cell marker/antigen 1.1; pSTAT5 - ; TCR-beta – T cell receptor-beta.
[b]Reactivity abbreviations: Ms-mouse; H-human; Rh-Rhesus monkey; Sh-sheep.
[c]Conjugate abbreviations: APC-Allophycocyanin, FITC-fluorescein isothiocyanate, PE-phycoerythrin; PE/Cy7-phycoerythrin/Cyanine 7.

2.2.2 Buffer and Culture Media

HyClone Roswell Park Memorial Institute 1640 medium (RPMI-1640) was purchased from (GE Healthcare Lifesciences catalogue #: SH30027.01). Ammonium-Chloride-Potassium (ACK) lysis buffer was made in house. FACS staining buffer (SB) was made in house (Phosphate buffer saline (PBS) pH 7.4 containing 2% fetal bovine serum). For cell surface staining, the fixation buffer was made in house (2% paraformaldehyde in PBS pH 7.4). For intracellular staining of Ki-67, Foxp3 Transcription Factor Staining Buffer Set (cat# 00552300) was purchased from eBioscience (San Diego, CA, USA). Briefly, to fix the cells 1 part of Foxp3 - Fixation Concentrate (cat# 00512343) was diluted into 3 parts of the Fixation/Permeabilization Diluent (cat# 00522356). For permeabilization, 1X Permeabilization buffer was made by diluting 10X Permeabilization concentrate (cat# 00833356) with deionized water. Sterile PBS used for mouse treatments was purchased from MULTICELL, cat# 311425CL.

2.2.3 Cytokines

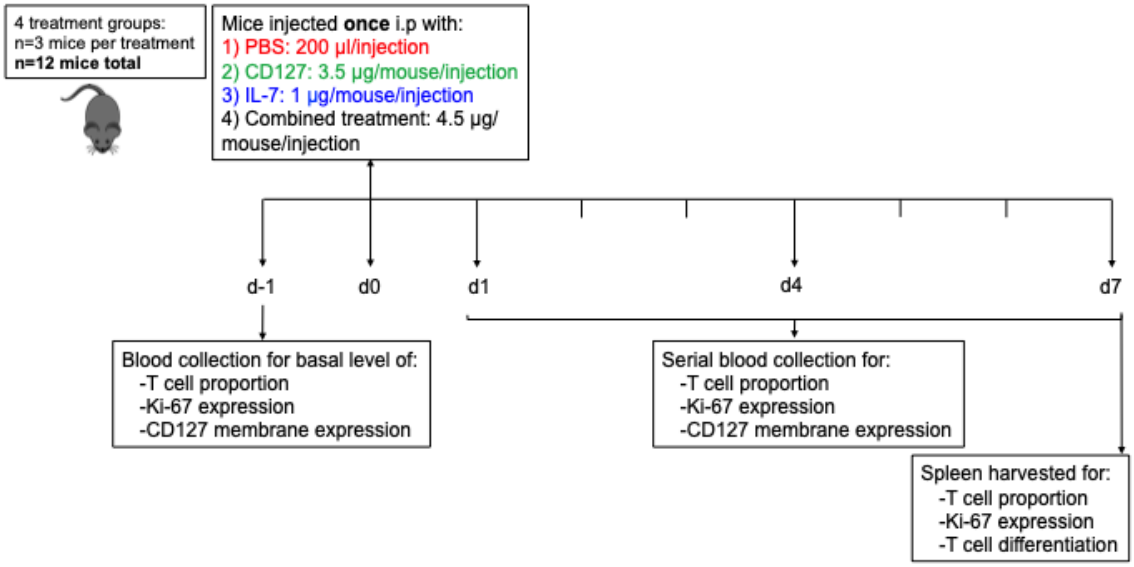
For mouse treatments, recombinant murine IL-7 (catalog #: 217-17; endotoxin level: less than 0.01 ng/mg cytokine as determined by the LAL assay) was purchased from PeproTech (Rocky Hill, USA). IL-7Ra-Fc chimeric molecule (catalogue #: 747-MR-050; endotoxin level: less than 1.0 Endotoxin Units/mg of protein by LAL method) was purchased from R&D Systems (Minneapolis, USA). For cytokines and soluble receptors quantification, AimPlex™ Mouse IL-7 Group 2 Analyte kit (cat# B211251) and AimPlex™ Mouse IL-7R/IL-7Rα/sCD127 Group 3 Analyte kit (cat# B21338) were purchased from YSL Biosciences Development Co. (Pomona, CA, USA).

2.3 Mouse treatments

2.3.1 Recombinant Murine IL-7 and sCD127 treatment of healthy mice

Mice were injected intraperitoneally (i.p) with murine IL-7 protein and mouse IL-7 Rα/CD127 Fc Chimera protein - that mimics the natural form of sCD127, either alone or in a combination. To allow the complex to form, right before administering the treatment, IL-7 and sCD127 were mixed at the indicated concentrations and incubated for 30 minutes (mins) at 37°C. This protocol was adapted from previous reports (153, 159). Two different approaches were used to treat the mice. The first approach was to use a dose of IL-7 and sCD127 that are in excess of their endogenous levels, and with an equal molar ratio for the combination treatment. In this approach, mice were divided into 4 groups and healthy mice were injected once with either 200 µl PBS alone (vehicle control), 1 µg of IL-7, 3.5 µg of sCD127, or 4.5 µg of IL-7 complexed with sCD127 (1 µg of IL-7 complexed to 3.5 µg of sCD127). In the second approach, mice were treated for 5 consecutive days. In this case, each mouse was treated either with 200 µl PBS alone, 5 µg of IL-7, 5 µg of sCD127, or a 10 µg complex of both treatments (5 µg of IL-7 complexed to 5 µg of sCD127). Of note, all treatment injections were diluted in sterile PBS (MULTICELL) and the total volume for each injection was kept consistent. The timeline of the experiments performed in healthy mice for both approaches can be found in **Figure 2-1A and B**.

A


4 treatment groups:
n=3 mice per treatment
n=12 mice total
Mice injected once i.p with:
1) PBS: 200 µl/injection
2) CD127: 3.5 µg/mouse/injection
3) IL-7: 1 µg/mouse/injection
4) Combined treatment: 4.5 µg/ mouse/injection
d-1
d0
d1
d4
d7
Blood collection for basal level of:
-T cell proportion
-Ki-67 expression
-CD127 membrane expression
Serial blood collection for:
-T cell proportion
-Ki-67 expression
-CD127 membrane expression
Spleen harvested for:
-T cell proportion
-Ki-67 expression
-T cell differentiation

B

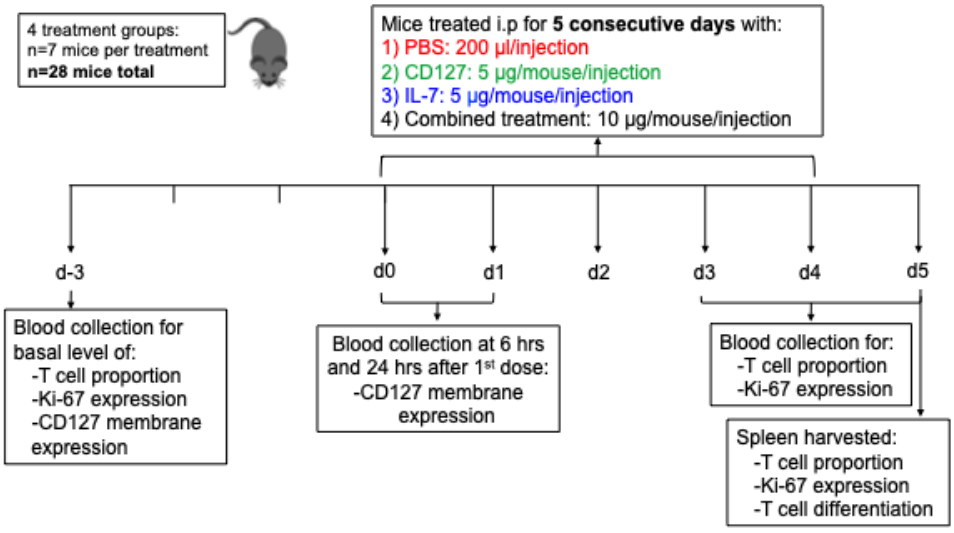

4 treatment groups:
n=7 mice per treatment
n=28 mice total
Mice treated i.p for 5 consecutive days with:
1) PBS: 200 µl/injection
2) CD127: 5 µg/mouse/injection
3) IL-7: 5 µg/mouse/injection
4) Combined treatment: 10 µg/mouse/injection
d-3
d0
d1
d2
d3
d4
d5
Blood collection for basal level of:
-T cell proportion
-Ki-67 expression
-CD127 membrane expression
Blood collection at 6 hrs and 24 hrs after 1st dose:
-CD127 membrane expression
Blood collection for:
-T cell proportion
-Ki-67 expression
Spleen harvested:
-T cell proportion
-Ki-67 expression
-T cell differentiation

Figure 2-1: Experimental timelines for experiments evaluating the effect of sCD127 and IL-7 treatments on blood T cell homeostasis in healthy C57BL/6 mice. A) Experimental timeline for approach #1 in healthy mice. Twelve healthy C57BL/6 were randomly allocated to one of four groups and i.p. injected on day 0 (d0) with either: 1) a single dose of soluble (3.5 μg) CD127; 2) a single equal molar dose (1 μg) of IL-7; 3) sCD127 complexed with IL-7 (3.5 μg sCD127 pre-incubated with 1 μg of IL-7 for 30 mins at 37°C); or 4) PBS (vehicle control). **B)** Experiment timeline for approach #2 in healthy mice. Healthy C57BL/6 mice received five consecutive days of i.p. injections of a high dose (5 μg) of soluble CD127 and/or equal mass of IL-7 and were compared mice receiving i.p. injections of PBS (vehicle control). Total n=28 mice.

2.3.2 *In-vivo* T cell depletion

For *in vivo* T cell depletion, B6 mice were administered 150 μg of both anti-CD4- (Clone GK1.5) and anti-CD8 (Clone YTS 169.4) depleting antibodies that were diluted in PBS i.p. on day 0 (d0). Blood was collected from mice on d1 to confirm T cell depletion by flow cytometry. Consequently, mice were bled weekly for 6 weeks to demonstrate the kinetics of T cell reconstitution following T cell depletion (from d7 to d63). A detailed description of the experiment timeline can be found in **Figure 2-2**.

2.3.3 Recombinant Murine IL-7 and sCD127 treatment of T cell depleted mice

The T cell depleted mice described in Section 2.3.2 were subsequently subjected to IL-7 and sCD127 treatments using the two approaches described in Section 2.3.1 with a few differences. In the first approach, the T cell depleted mice were injected twice with either 200 μl PBS alone (vehicle control), 1 μg of IL-7, 3.5 μg of sCD127, or 4.5 μg of IL-7 complexed with sCD127 (1 μg of IL-7 complexed to 3.5 μg of sCD127) at d6 and d13 following T cell depletion (**Figure 2-3A**). For the second approach, the T cell depleted mice were injected for five consecutive days with either 200 μl PBS alone, 5 μg of IL-7, 5 μg of sCD127, or a 10 μg complex of both treatments (5 μg of IL-7 complexed to 5 μg of sCD127) on d24-d28 after T cell depletion (**Figure 2-3B**). Of note, T cell depleting antibodies were injected at d0 in both cases.

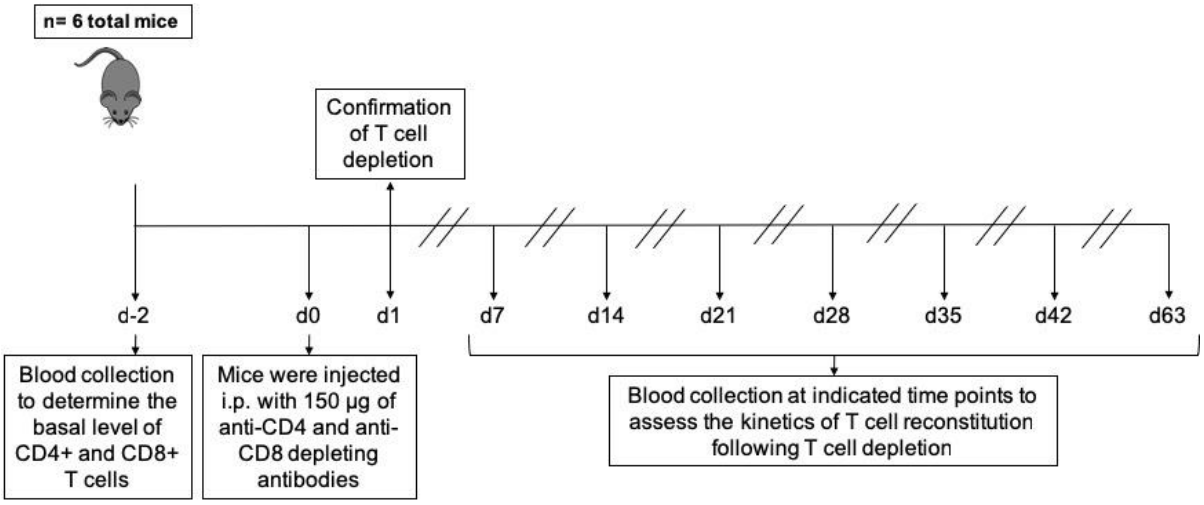
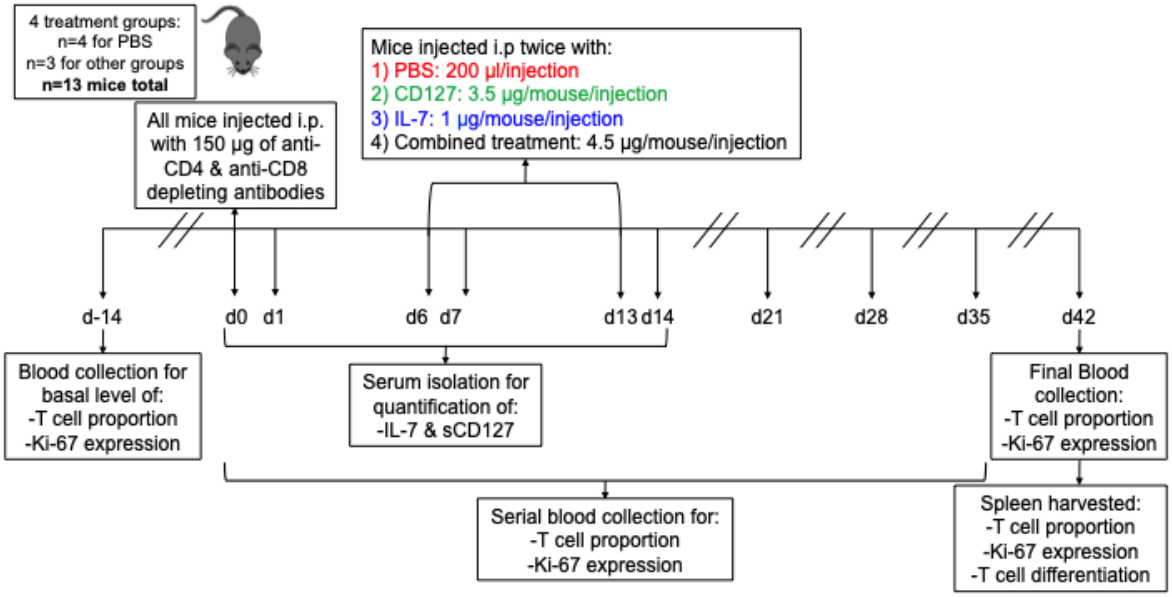

n= 6 total mice
Confirmation of T cell depletion
d-2
d0
d1
d7
d14
d21
d28
d35
d42
d63
Blood collection to determine the basal level of CD4+ and CD8+ T cells
Mice were injected i.p. with 150 µg of anti-CD4 and anti-CD8 depleting antibodies
Blood collection at indicated time points to assess the kinetics of T cell reconstitution following T cell depletion

Figure 2-2: Experimental timeline for the T cell depletion optimization experiments in healthy C57BL/6 mice. Six healthy age-matched C57BL/6 mice had blood drawn two days (d-2) before starting the experiment. On day 0 (d0), each mouse was i.p. injected with a 150 µg dose of both anti-CD4 and anti-CD8 depleting antibodies. On d1, T cell depletion was confirmed by analyzing the blood T cell proportion by flow cytometry as described in section 3.2.1. Blood was taken from each mouse at the indicated time points, and stained with CD3, CD4, and CD8 antibodies to assess the kinetics of T cell reconstitution up to d63.

A


4 treatment groups:
n=4 for PBS
n=3 for other groups
n=13 mice total
All mice injected i.p. with 150 µg of anti-CD4 & anti-CD8 depleting antibodies
Mice injected i.p twice with:
1) PBS: 200 µl/injection
2) CD127: 3.5 µg/mouse/injection
3) IL-7: 1 µg/mouse/injection
4) Combined treatment: 4.5 µg/mouse/injection
d-14
d0
d1
d6
d7
d13
d14
d21
d28
d35
d42
Blood collection for basal level of:
-T cell proportion
-Ki-67 expression
Serum isolation for quantification of:
-IL-7 & sCD127
Final Blood collection:
-T cell proportion
-Ki-67 expression
Serial blood collection for:
-T cell proportion
-Ki-67 expression
Spleen harvested:
-T cell proportion
-Ki-67 expression
-T cell differentiation

B

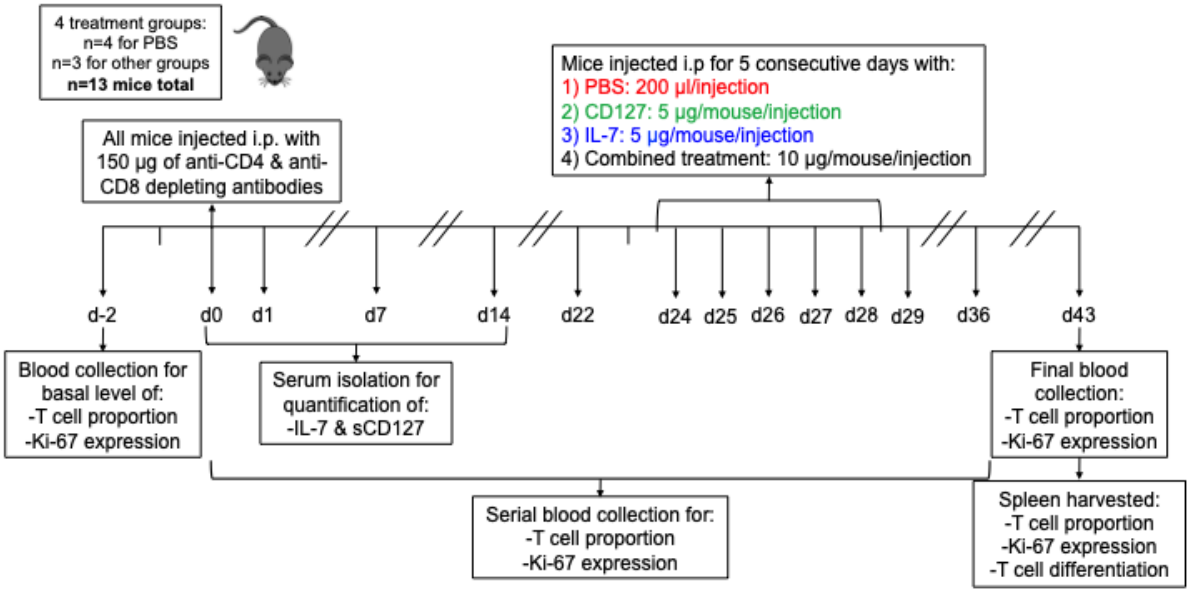

4 treatment groups:
n=4 for PBS
n=3 for other groups
n=13 mice total
All mice injected i.p. with 150 µg of anti-CD4 & anti-CD8 depleting antibodies
Mice injected i.p for 5 consecutive days with:
1) PBS: 200 µl/injection
2) CD127: 5 µg/mouse/injection
3) IL-7: 5 µg/mouse/injection
4) Combined treatment: 10 µg/mouse/injection
d-2
d0
d1
d7
d14
d22
d24
d25
d26
d27
d28
d29
d36
d43
Blood collection for basal level of:
-T cell proportion
-Ki-67 expression
Serum isolation for quantification of:
-IL-7 & sCD127
Final blood collection:
-T cell proportion
-Ki-67 expression
Serial blood collection for:
-T cell proportion
-Ki-67 expression
Spleen harvested:
-T cell proportion
-Ki-67 expression
-T cell differentiation

Figure 2-3: Experimental timelines for experiments evaluating the effect of sCD127 and IL-7 treatments on blood T cell homeostasis in T cell depleted C57BL/6 mice. A) Experimental timeline for approach #1 in T cell depleted mice. T cell depleted mice were i.p. injected with two doses of soluble CD127 and/or IL-7 on two consecutive days and compared to those injected with PBS (vehicle control). Total n=13 mice (n=4 PBS, n=3 for other groups). **B)** Experiment timeline for approach #2 in T cell depleted mice. T cell depleted mice were i.p. injected with five consecutive daily doses of soluble CD127 and/or IL-7 and compared to T cell depleted mice injected with PBS (vehicle control). Total n=16 mice

2.4 Cell Isolation

2.4.1 Peripheral blood mononuclear cell isolation

Approximately 40 µl of blood was collected in heparin-coated capillary tubes (BD Vacutainer, cat# 026896) via a saphenous vein of experimental mice at appropriate time points. The blood samples were kept on ice throughout the procedures. After collection, blood was then mixed in 1 ml of PBS in a 15 ml conical centrifuge tube (Thermo Scientific, cat# 339650), and washed by centrifugation for 5 mins at 4°C and at 652 xg. The supernatant was discarded. The Red blood cells (RBCs) were then lysed in 1 ml ACK lysis buffer for 1 min or until the solution began to appear transparent at room temperature (RT). Then, 9 ml of RPMI-1640 was added immediately, filtered through a 200 micron nylon mesh (Elko Filtering, cat# 0320045) cell strainer and washed by centrifugation once for 5 mins at 4°C and at 652 xg. The cells were then resuspended in 2 ml media, counted on a hemocytometer, washed by centrifugation again for 5 mins at 4°C and at 652 xg, and then resuspended in SB for further analysis. The number of total peripheral blood mononuclear cells (PBMCs) obtained per 40 µl of mouse blood yields 1-2 X 10^5 cells and approximately 40% are T cells.

2.4.2 Splenocyte isolation

Spleens were harvested from experimental mice at the last time point of an experiment when indicated. The cells are then transferred to 6-well normal tissue culture plate (Greiner Bio-One, cat# 07000079) containing 5 ml RPMI-1640 media per well. To obtain a single cell suspension, spleens were gently dissociated in media and pushed through a 70 µm nylon cell strainers (Bio Basic Inc, cat # SP104181) using the end of a 3 ml syringe plunger. Splenocytes, a mixed population of all cells isolated from the spleen including T cells, B cells, DCs and

macrophages, were then transferred to a 15 ml conical centrifuge tube and lysed in 1 ml ACK lysis buffer for 30 seconds at RT. Then, 14 ml of media was added to dilute the lysis buffer, and centrifuged at 290 xg for 10 mins at 4°C. The cells were then resuspended in 10 ml media, filtered through a nylon mesh, counted on a hemocytometer, and washed once in RPMI-1640 media by centrifugation at 290 xg for 10 mins at 4°C. From this method, we expect yields of 5-10 X 10^7 total splenocytes per spleen and of those 50% cells are T cells. Cells were then resuspended in media for subsequent cell staining and further analysis at assay-specific cell densities.

2.5 Flow cytometry

2.5.1 Cell surface marker expression

Cell suspensions prepared from mouse blood or mouse spleens at appropriate time points were resuspended in SB at a density of 1 X 10^6 cells/well in a V-bottom 96-well plate (Thermo Scientific, cat# 12556008). Cells were spun down at 453 xg for 4 mins at 4°C and subsequently stained with a cocktail of directly conjugated monoclonal antibodies (mAbs) (0.2 µl of each antibody/10^6 cells) in 40 µl of SB and incubated at 4°C for 25 mins in the dark. After staining, cells were washed with SB, fixed with 2% paraformaldehyde (PFA) for 10 mins at 4°C, and washed twice to remove PFA. Finally, cells were resuspended in 300 µl of SB and processed within 24 hours. Samples in 96 well plates were run on the LSRFortessa™ or FACSCelesta™ at a sample flow rate of approximately 60 µL/min and approximately 300 to 600 events per second (both BD Biosciences). Data was analyzed using Kaluza software v2.0 (Beckman Coulter).

The phenotype of the cells was determined using the following fluorescent mAbs against cell surface or viability markers including: anti-CD3, anti-CD4, and anti-CD8α for T cells; anti-NK1.1 for NK and NKT cells; fixable viability stain for dead cells; anti-CD127 for Membrane surface expression of CD127; and anti-CD62L and anti-CD44 for T cell subset identification. More information about staining panels and controls used to examine the cells can be found in **Table 7-1**.

2.5.2 Intracellular staining for Ki-67

For intracellular staining, surface staining was completed first, and then the cells were fixed for 12-16 hours at 4°C in the dark. The cells were handled for the subsequent steps at RT. Briefly, cells were permeabilized twice by adding 150 µl of perm buffer and washed once by centrifugation at 453 xg for 4 mins at 4°C. The cells were then stained with V450-conjugated anti-mouse monoclonal Ki-67 antibodies (0.2 µl/10^6 cells) in 40 µl of perm buffer and incubated at RT for 30 mins in the dark. The cells were washed twice with perm buffer and resuspended in 300 µl of SB for further flow cytometric analysis.

2.5.3 Measurement of plasma IL-7 and sCD127 levels

For the evaluation of serum cytokine levels, the AimPlex™ mouse IL-7 and the AimPlex™ mouse IL-7Rα/sCD127 Single-Plex kits were used. The samples were prepared according to the manufacturer's instructions. Briefly, 11 µl of a mixture of capture bead coated with antibody specific (either for IL-7 or sCD127) was first mixed with equal volume of either sample or standard, and incubated on an orbital shaker, at RT in the dark for 1 hour. Then, biotinylated detection antibodies (11 µl) were added to form sandwich complexes and

incubated in the dark for 30 mins on the shaker. Next, streptavidin-PE was added to the samples and incubated for 20 mins Subsequently, the samples were washed in 100 µl washing buffer, spun down at 163 xg for 5 mins After washing, the samples were resuspended in 300 µl of reading buffer, acquired using an LSRFortessa™, and analyzed using the BD FCAP Array Software. The cytokine concentration in pg/ml was calculated based on the standard curves.

2.6 Calculation of absolute T lymphocyte subset count

In order to calculate mice blood and spleen cell number, the following calculations were used:

- **Blood cell count:**

Total cell number of specified T cell gate counted by flow cytometry = A

Total blood volume collected = 40 µl

Total volume of SB used for flow acquisition = 150 µl

Volume acquired for each sample by flow = 100 µl

Absolute number of specified T cell subset / 40 µl of blood = A X $\frac{150}{100}$ = B

Absolute number of specified T cell subset / ml of blood = B X $\frac{1000}{40}$

- **Spleen cell count:**

Cell number counted by hemocytometer = A

Percentage of T cell subset gate of total live cells = B

Absolute number of specified T cell subset = A X B

2.7 Statistical analysis

All statistical analyses were determined using GraphPad Prism 5.0 software (San Diego, CA). Statistical significance was calculated by performing a One-way analysis of variance (ANOVA) followed by unpaired parametric Student's t-test. Values with a cut off P value less than 0.05 were considered significant. Data are presented as mean ± standard deviation (SD), and P values are represented as not significant (ns), *P< 0.05, ** P< 0.01, *** P< 0.001.

3. Chapter 3: Results

3.1 Determine the effect of sCD127 with or without IL-7 on T cell homeostasis in healthy mice

3.1.1 Safety of sCD127 and IL-7 treatment in our *in vivo* system

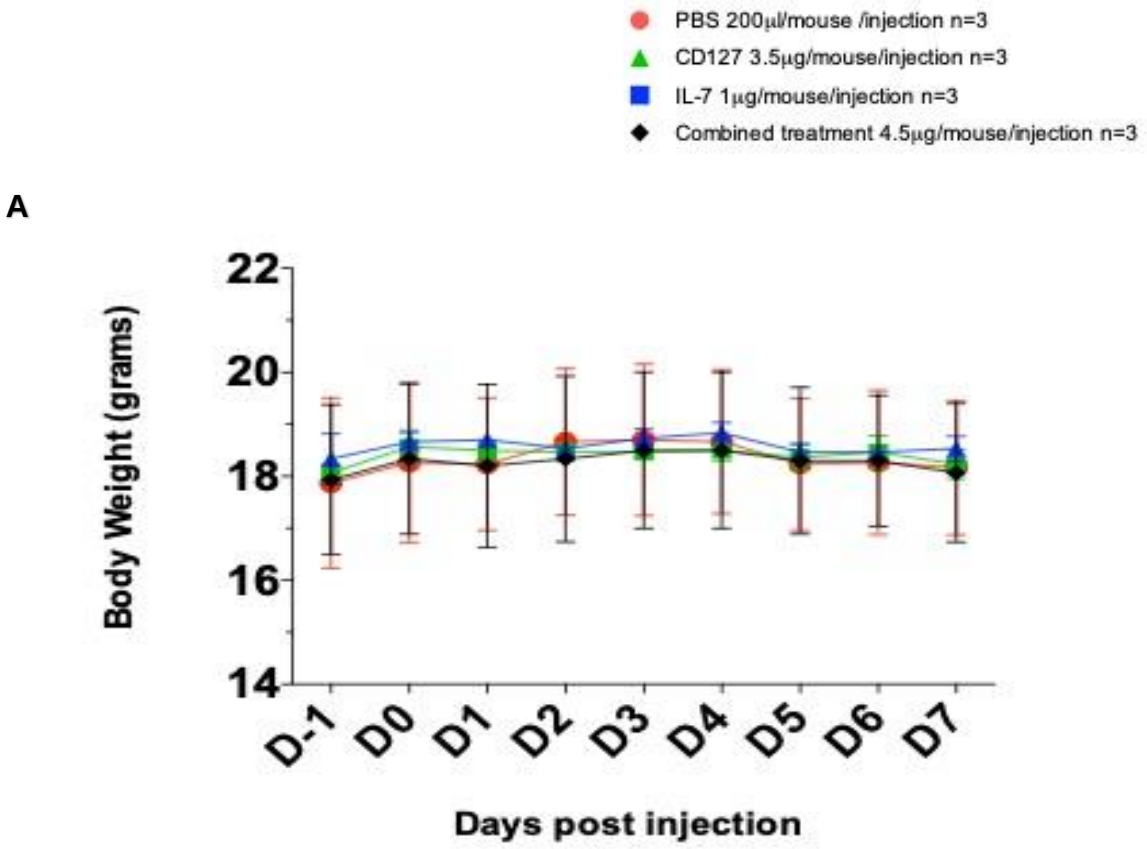
Since little is known about sCD127 use in *in vivo* experiments, we assessed the safety of administering soluble recombinant mouse IL-7-R alpha/CD127 Fc Chimera Protein (R&D Systems) to healthy mice by first measuring mouse body weight. A total of 12 mice were used in this experiment, and the mice were divided into four groups. Each group received a single i.p. injection of either: sCD127 alone (3.5 μg); IL-7 alone (1 μg); the combined dose of both sCD127 and IL-7 (pre-incubated at 37C^{o} for 30 mins to complex proteins); or PBS (vehicle control). We demonstrated that all mice tolerated the treatments and there was no noticeable weight loss in any mouse on the indicated treatments **Figure 3-1A and B**. Moreover, even after we increased the dosage time and concentration of treatments as outlined (See **Figure 2-1**B), we did not observe any toxic effects as determined by change in mouse body weight **Figure 7-1**.

3.1.2 The effect of IL-7 and sCD127 treatment on blood T cell homeostasis in healthy mice

3.1.2.1 Treatment with sCD127 did not inhibit IL-7 induced downregulation of surface CD127 expression on T cells

To determine whether the treatments act directly on T cells, the down-regulation of surface CD127 expression on $CD4^{+}$ and $CD8^{+}$ T cells, a known marker for signal transduction of IL-7 (160), was examined by flow cytometry. PBMCs were isolated from healthy mice at

time points mentioned above, and stained with antibodies against CD127, CD3, CD4, and CD8. We found that the proportion of $CD4^+$ and $CD8^+$ T cells expressing CD127 was approximately around on ~85% in both $CD4^+$ and $CD8^+$ T cells before treatment. Treatment with IL-7 with or without sCD127 significantly reduced the expression of CD127 on $CD4^+$ or $CD8^+$ T cells 6 hours following treatment initiation as compared to the PBS on treated mice at the same time point (**Figure 3-2**). In the case of $CD4^+$ T cells, CD127 was expressed on 45% mean ± 5% SD and 50% ± 10% in IL-7 and combined therapy treated groups, respectively compared to 98% ± 1% observed in PBS treated one. While the proportion of $CD8^+$ T cells expressing CD127 was approximately 38% ± 4% and 42% ± 12% in IL-7 and combined therapy treated groups, respectively relative to 99% ± 1% in PBS treated mice. Treatment of sCD127 alone did not alter the percentage of CD4 and CD8 expressing CD127 as compared to the PBS treated mice **Figure 3-2A** and **B**. Interestingly, the effect on CD127 downregulation was transient and CD127 expression returned to basal levels 24 hours after treatment.


PBS 200µl/mouse /injection n=3
CD127 3.5µg/mouse/injection n=3
IL-7 1µg/mouse/injection n=3
Combined treatment 4.5µg/mouse/injection n=3
A
Body Weight (grams)
22
20
18
16
14
D-1
D0
D1
D2
D3
D4
D5
D6
D7
Days post injection

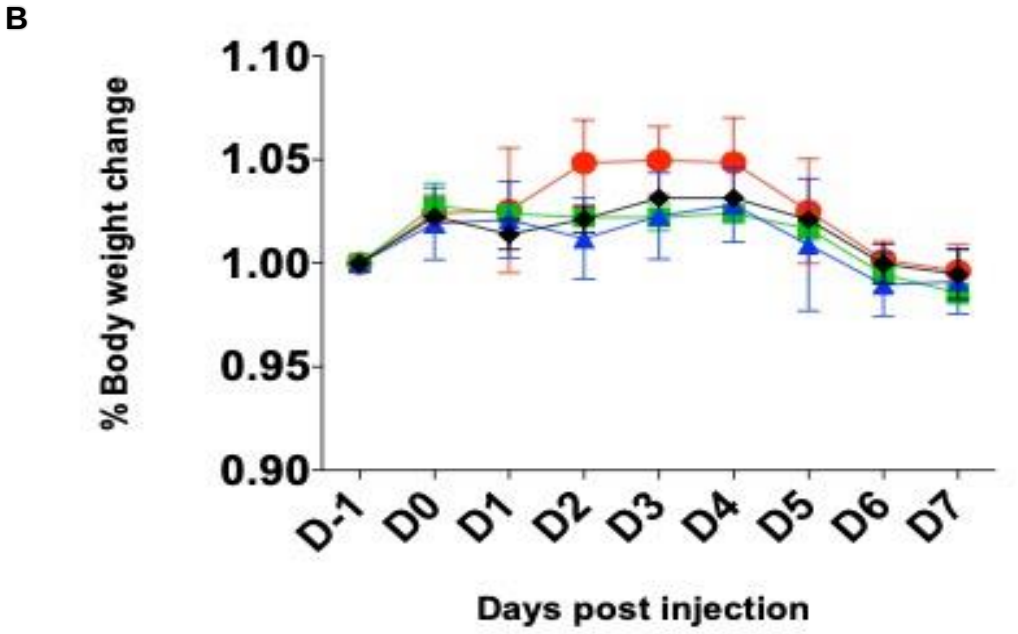

B
% Body weight change
1.10
1.05
1.00
0.95
0.90
D-1
D0
D1
D2
D3
D4
D5
D6
D7
Days post injection

Figure 3-1: The effect of sCD127 and IL-7 treatments on mouse body weight, total n=12 mice. Daily body weight (in grams) of mice in each treatment group was recorded at baseline (d-1) and after treatment (d0-d7) with sCD127, IL-7, or sCD127 complexed with IL-7, or PBS alone. **A)** Mean body weight of mice in each treatment group (in grams) over the course of the study treatment ± SD. **B)** Changes in daily body weight were normalized relative to the starting weight for each individual mouse and then the mean for each treatment group was reported as the body weight change relative to baseline = 1.0 (baseline weight at d-1 = 1.0) ± SD. There were no significant differences in body weight between baseline and treatments or control (PBS) and other treatments.

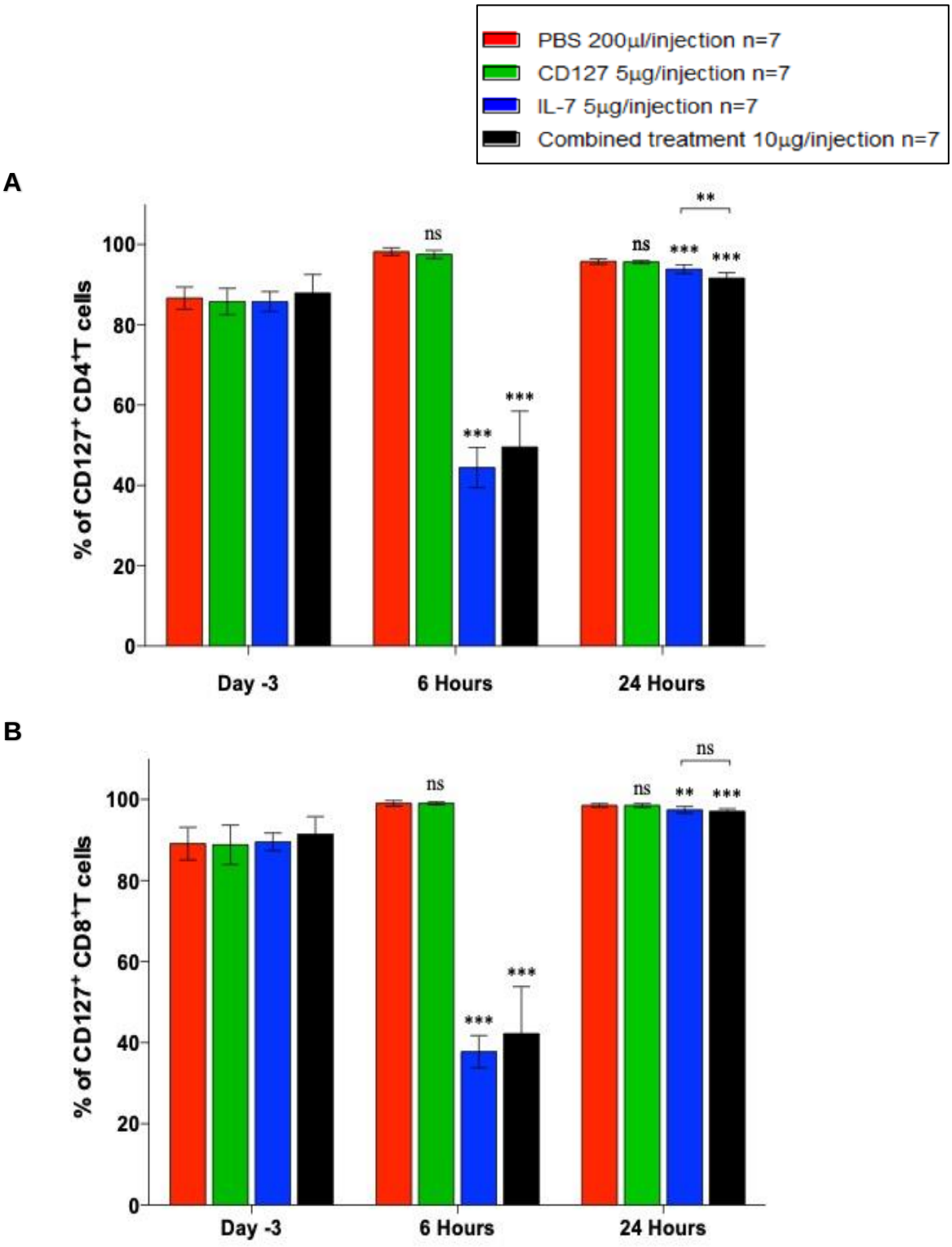
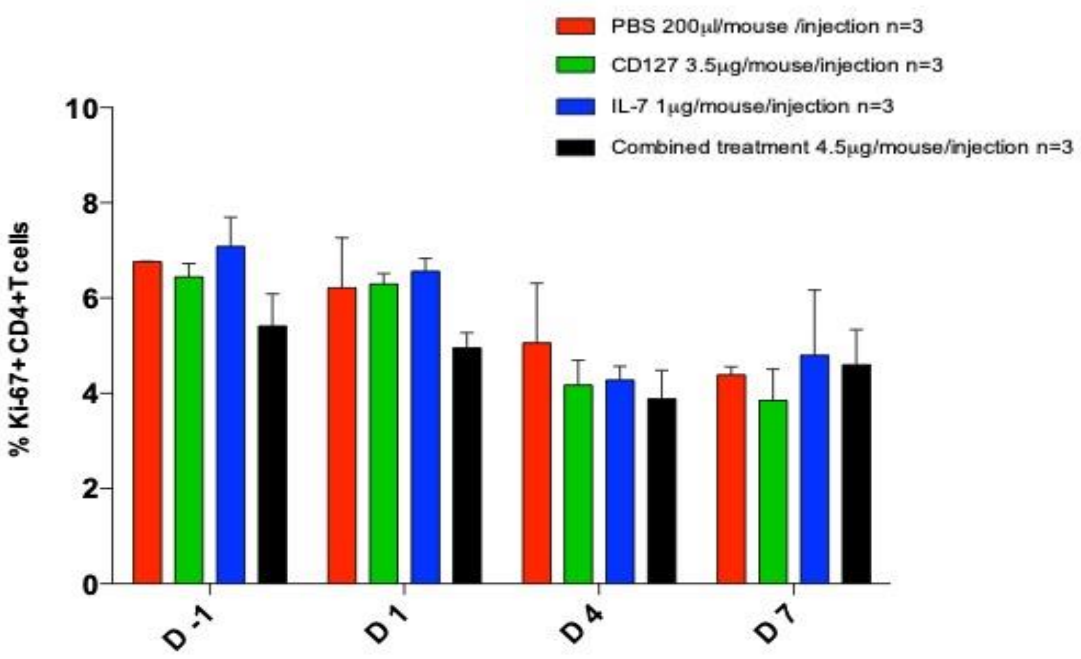

PBS 200µl/injection n=7
CD127 5µg/injection n=7
IL-7 5µg/injection n=7
Combined treatment 10µg/injection n=7
A
% of CD127+ CD4+T cells
100
80
60
40
20
0
ns
ns
***
***
***
***
**
Day -3
6 Hours
24 Hours
B
% of CD127+ CD8+T cells
100
80
60
40
20
0
ns
ns
***
***
**
***
ns
Day -3
6 Hours
24 Hours

Figure 3-2: The effect of sCD127 and IL-7 treatment on blood T cell homeostasis in healthy C57BL/6 mice. Mice were divided into 4 groups, and treated for 5 consecutive days either with sCD127 alone, IL-7 alone, a combined dose of sCD127 and IL-7 (pre-incubated at 37°C for 30 mins), or PBS. The graphs show the kinetics of CD127 expression on $CD4^+$ **(A)** and $CD8^+$ **(B)** T cells. Data are pooled from two independent experiments, n=7 mice per group. Data represent mean ± SD and statistical analysis was completed using a one-way ANOVA for more than two groups, and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$) or significant, with P values grouped as follows: * $P<0.05$, ** $P<0.01$, or *** $P<0.001$. An asterisk above a column bar represents a statistically significant difference between the indicated treatment and PBS-treated mice at the same time point. An asterisk above a line connecting two column bars shows a statistically significant difference between the two indicated treatments.

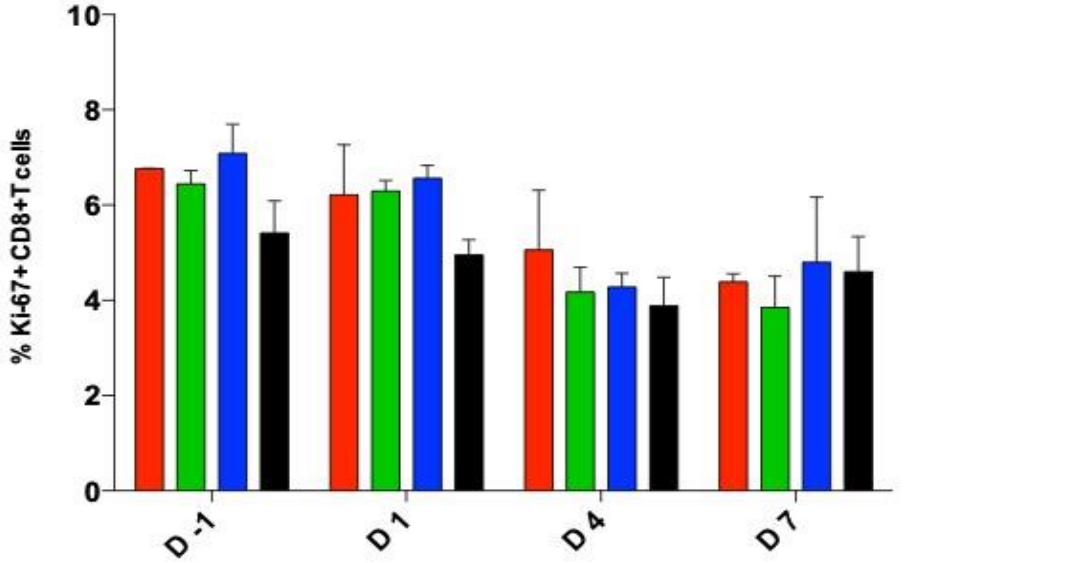
3.1.2.2 Ki-67 expression in T cells increases following sCD127 and IL-7 treatment

To test if sCD127 would enhance the effect on IL-7-mediated T cell proliferation, mice were bled at different time points to assess the change in expression of the nuclear antigen Ki-67, a marker of T cell proliferation, by flow cytometry. In the basal state, the percentage of Ki-67+ $CD4^+$ and $CD8^+$ T cells was found to be consistently below 7%. Our initial investigation demonstrated that a single dose of IL-7 as described above (See **Figure 2-1A**), had no significant impact on T cell proliferation in peripheral blood **Figure 3-3A and B**. Therefore, we increased the dosage time and concentration as described in the Methods (See **Figure 2-1B**). Mice were injected by the i.p. route with a higher dose of the indicated treatment for 5 consecutive days and bled at d3 and d5 post-injection to assess the proliferation profile of the T cells. Our results demonstrated that IL-7 treatment significantly increased the number of both Ki-67-positive $CD4^+$ and $CD8^+$ T cells compared to the PBS-treated mice at the same time point. By d3 of IL-7 treatment, 7% and 16% of the circulating $CD4^+$ and $CD8^+$ T cells were Ki-67+ (respectively), with a robust effect on T cell proliferation observed at d5 in which 20% of $CD4^+$ T cells and 45% of $CD8^+$ T cells were Ki-67+ relative to the 5% in PBS-only treated mice (**Figure 3-3C, D and appendix figure 7.2**). Interestingly, combined treatment with sCD127 further enhanced the effect of IL-7 on T cell proliferation, at both d3 and d5 **Figure 3-3C and D**. There was no apparent difference in the percentage of Ki-67 when mice were treated with sCD127 alone relative to PBS. Of interest, the increase in Ki-67 was considerably greater in $CD8^+$ T cells than in $CD4^+$ T cells (**Figure 3-3C and D**).

A


PBS 200µl/mouse /injection n=3
CD127 3.5µg/mouse/injection n=3
IL-7 1µg/mouse/injection n=3
Combined treatment 4.5µg/mouse/injection n=3
% Ki-67+CD4+T cells
10
8
6
4
2
0
D -1
D 1
D 4
D 7

B


% Ki-67+CD8+T cells
10
8
6
4
2
0
D -1
D 1
D 4
D 7

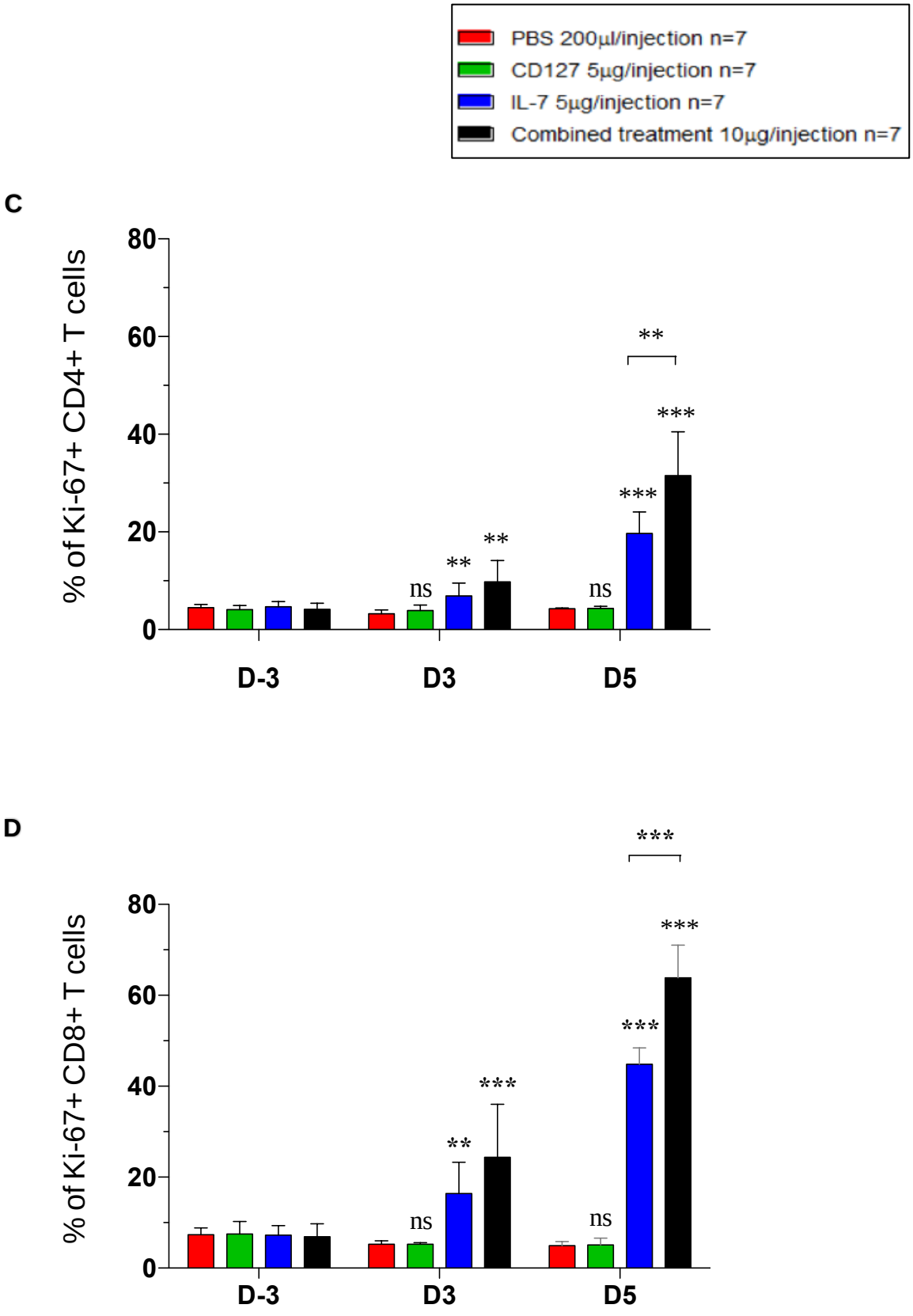

PBS 200µl/injection n=7
CD127 5µg/injection n=7
IL-7 5µg/injection n=7
Combined treatment 10µg/injection n=7
C
% of Ki-67+ CD4+ T cells
80
60
40
20
0
**
***
***
**
**
ns
ns
D-3
D3
D5
D
% of Ki-67+ CD8+ T cells
80
60
40
20
0
***
***
***
**
ns
ns
D-3
D3
D5

Figure 3-3: The effect of sCD127 treatment on blood T cell proliferation in healthy C57BL/6 mice. A/B) The effect of low, single dose sCD127 and/or IL-7 on T cell proliferation in the blood of healthy C57BL/6 mice. Mice were divided into 4 groups, and treated once with either 1 μg of IL-7, 3.5 μg of sCD127, 4.5 μg of IL-7 and sCD127 complexed (pre-incubated together for 30 mins at 37°C), or 200 μl PBS alone (vehicle control). The graphs show the frequency of Ki67+ cells amongst $CD4^+$ **(A)** and $CD8^+$ **(B)** T cells. n=3 mice per treatment group. **C/D)** The effect of five consecutive days of high dose sCD127 and/or IL-7 on T cell proliferation in the blood of healthy C57BL/6 mice. Mice were divided into 4 groups, and treated by i.p. injection for five consecutive days with either: 5 μg of IL-7, 5 μg of sCD127, 10 μg of both treatments complexed (pre-incubated for 30 mins at 37°C), or 200 μl of PBS alone (vehicle control). The graphs show the frequency of Ki67+ cells amongst $CD4^+$ **(C)** and $CD8^+$ **(D)** T cells. n=7 mice per treatment group. Data are pooled from two independent experiments. Data represent mean ± SD. Statistical analysis was completed using a one-way ANOVA for more than two groups, and a Student's two-tailed unpaired t-test was used for comparisons between two groups. Significant P values are listed as either *$P<0.05$, ** $P<0.05$, or ***$P<0.001$.

3.1.2.3 Combination of sCD127 and IL-7 enhances IL-7-mediated increase in $CD8^+$ T cells number, but not $CD4^+$ T cells

The ability of IL-7 and the combination therapy of sCD127 complexed to IL-7 to induce T cell proliferation suggested that the treatments would consequently increase T cell numbers. To demonstrate the effects of IL-7 and/or sCD127 treatments on T cell number, we treated healthy mice either with 5 μg of IL-7, 5 μg of sCD127, a combination of these two, or 200 μl PBS alone for 5 consecutive days. Total $CD4^+$ and $CD8^+$ T cell numbers in blood were measured at d5 of treatment. Mice treated with either IL-7 or combined therapy had increased T cell numbers relative to their PBS treated counterparts. However, the difference observed in $CD4^+$ T cell count did not reach a statistical significance with a total CD4 number ranging from approximately $7.5x10^5$ cells and $9.9x10^5$ cells per ml of blood in IL-7 and combined therapy respectively, compared to the $3.9x10^5$ cells per ml of blood in PBS treated mice **Figure 3-4A**. In contrast, total blood $CD8^+$ T cell count was significantly higher in the mice treated with IL-7 or combined therapy relative to control (PBS treated) mice. The average absolute number of $CD8^+$ T cells was $3.5x10^5$ cells/ml of blood and $5x10^5$ cells per ml of blood for IL-7 and combined therapy mice respectively, relative to $1.5x10^5$ $CD8^+$ T cells per ml of blood in PBS treated mice **Figure 3-4B.**

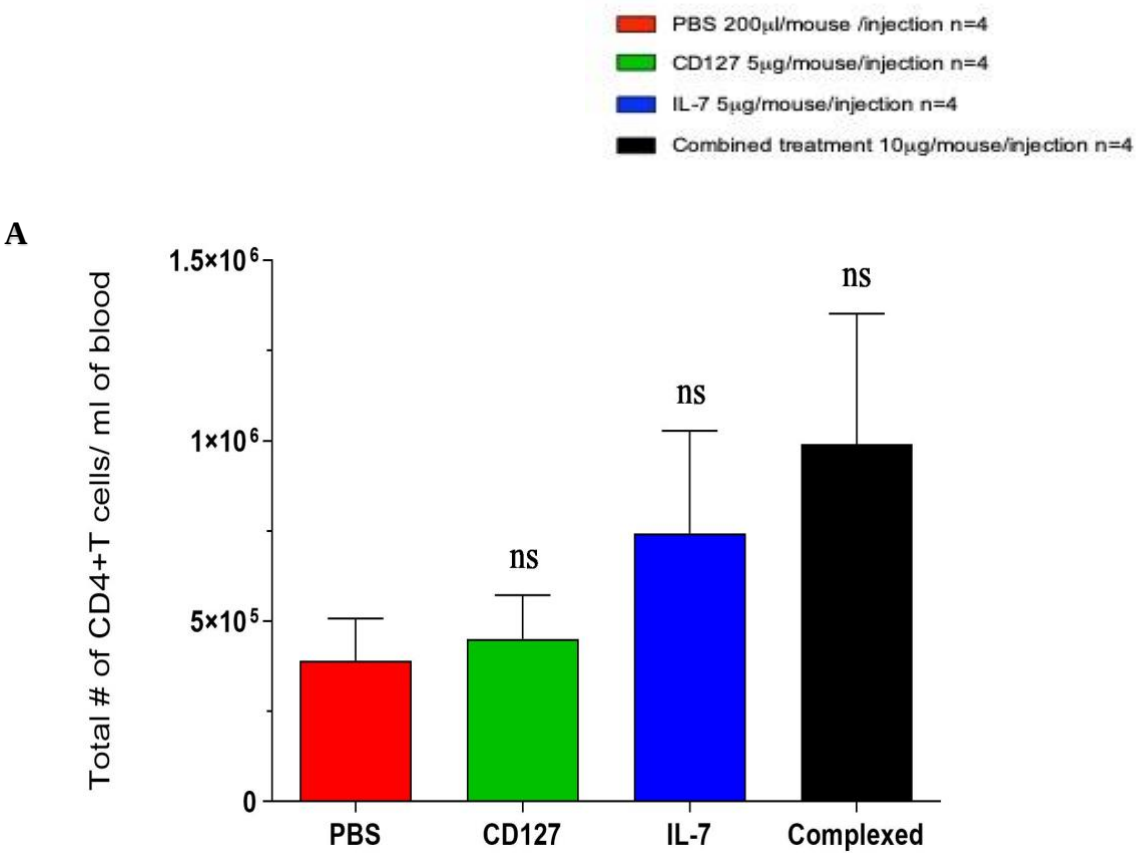

PBS 200µl/mouse /injection n=4
CD127 5µg/mouse/injection n=4
IL-7 5µg/mouse/injection n=4
Combined treatment 10µg/mouse/injection n=4
A
Total # of CD4+T cells/ ml of blood
1.5×10^6
1×10^6
5×10^5
0
ns
ns
ns
PBS
CD127
IL-7
Complexed
B
Total # of CD8+T cells/ ml of blood
8×10^5
6×10^5
4×10^5
2×10^5
0
ns
*
**
PBS
CD127
IL-7
Complexed

Figure 3-4: The effect of IL-7 and sCD127 treatment on T cell numbers in the blood of healthy C57BL/6 mice. The number of $CD4^+$ **(A)** or $CD8^+$ **(B)** T cells was measured in the blood of mice at d5, following five consecutive days i.p. injected with either: 5 μg of IL-7, 5 μg of sCD127, a complex of 5 μg of IL-7 with 5 μg of sCD127 (pre-incubated for 30 mins at 37°C) or PBS alone. Data represent mean ± SD. Statistical analysis was completed using one-way ANOVA for more than two groups, and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$) or significant with *$P<0.05$, **$P<0.01$, or ***$P<0.001$.

3.1.3 The effect of IL-7 and sCD127 treatment on spleen T cell homeostasis in healthy mice

3.1.3.1 Level of Ki-67 expression in T cells is significantly increased following sCD127 and IL-7 treatment in spleen

All mice were treated with i.p. injections for 5 consecutive days, and the spleens were harvested at d5 to assess the proliferation profile of T cells (**See Figure 2-1B**). Consistent with what was found in blood, the basal percentage of Ki-67+ $CD4^+$ or $CD8^+$ T cells was below 7%. Similar to the result observed in blood, the addition of sCD127 significantly augmented the ability of IL-7 to stimulate T cell proliferation in the spleen as indicated by elevated Ki-67 expression. Approximately 22% to 32% of the circulating $CD4^+$ were Ki-67+ following IL-7 and combined therapy respectively, relative to 6% of the cells in PBS treated mice. Interestingly, a more robust effect on T cell proliferation was observed in $CD8^+$ T cells in which 40% to 56% of the cells were positive for Ki-67 (in IL-7 or combined treatments respectively) compared to the 5% demonstrated in PBS treated mice. Moreover, there was significant enhancement of combined treatment with sCD127 compared to IL-7 alone in inducing T cell proliferation (**Figure 3-5A** for $CD4^+$ T cells, P value = 0.0413; for $CD8^+$ T cells, **Figure 3-5B**, P value = 0.0011). Similar to the Ki-67 results in blood, there was a tendency of high responsiveness of $CD8^+$ T cells toward IL-7 treatments compared to $CD4^+$ T cells **Figure 3-5A and B**.

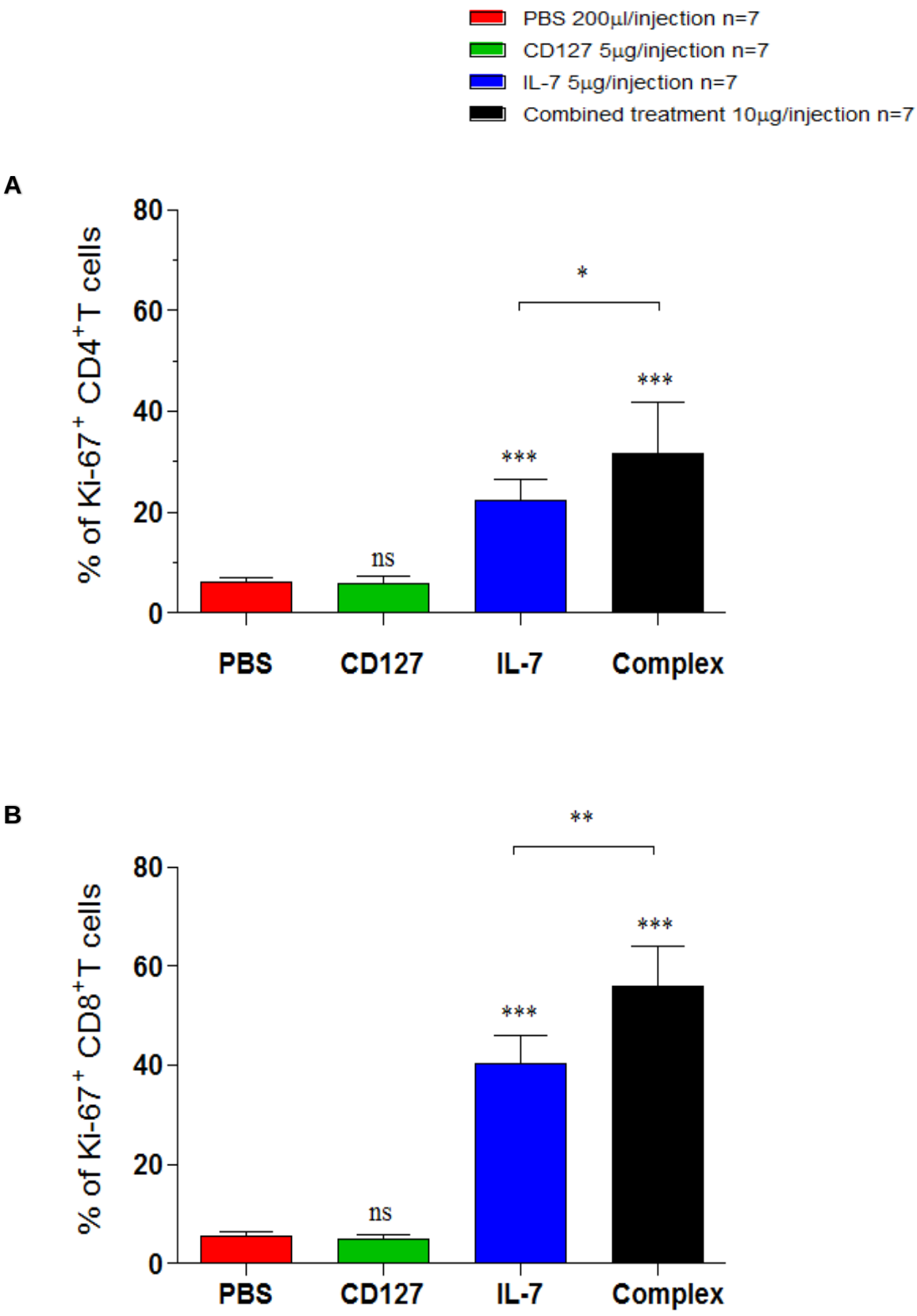
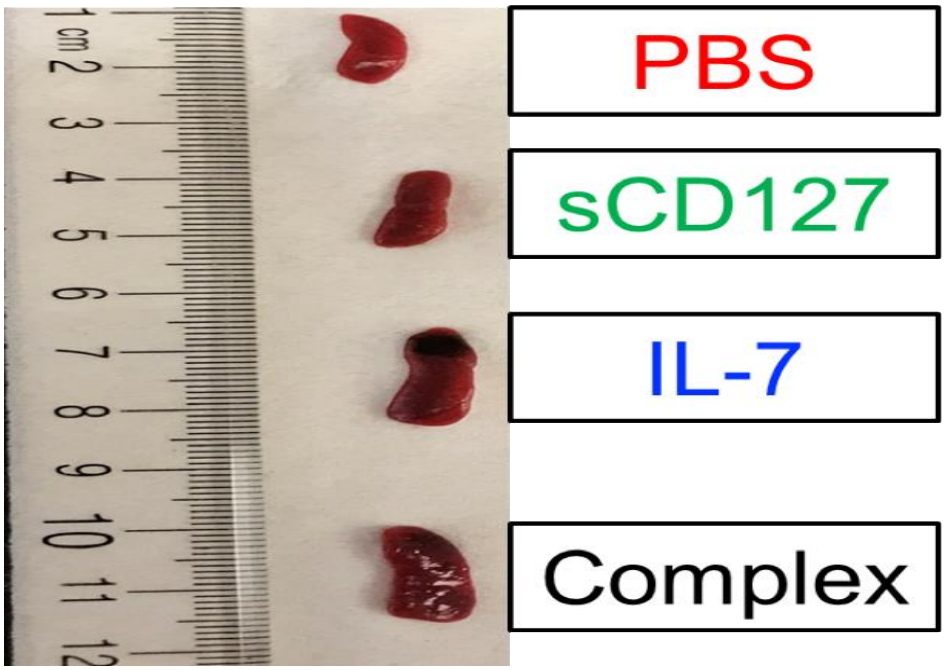

PBS 200µl/injection n=7
CD127 5µg/injection n=7
IL-7 5µg/injection n=7
Combined treatment 10µg/injection n=7
A
% of Ki-67+ CD4+T cells
80
60
40
20
0
*
***
***
ns
PBS
CD127
IL-7
Complex
B
% of Ki-67+ CD8+T cells
80
60
40
20
0
**
***
***
ns
PBS
CD127
IL-7
Complex

Figure 3-5: The effect of sCD127 treatment on spleen T cell proliferation in healthy C57BL/6 mice. The graphs show the frequency of Ki-67+ cells from $CD4^+$ **(A)** and $CD8^+$ **(B)** T cells isolated from the spleen. Data were pooled from two independent experiments, with a total of n=7 mice per treatment group. Mice were divided into 4 groups, treated for 5 consecutive day either with 5 μg of IL-7, 5 μg of sCD127, 5 μg of IL-7 complexed with 5 μg of sCD127 (pre-incubated for 30 mins at 37°C), or 200 μl of PBS alone. Data represent mean ± SD. Statistical analysis was completed using a one-way ANOVA for more than two groups and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$), or significant with *$P<0.05$, **$P<0.01$, or ***$P<0.001$.

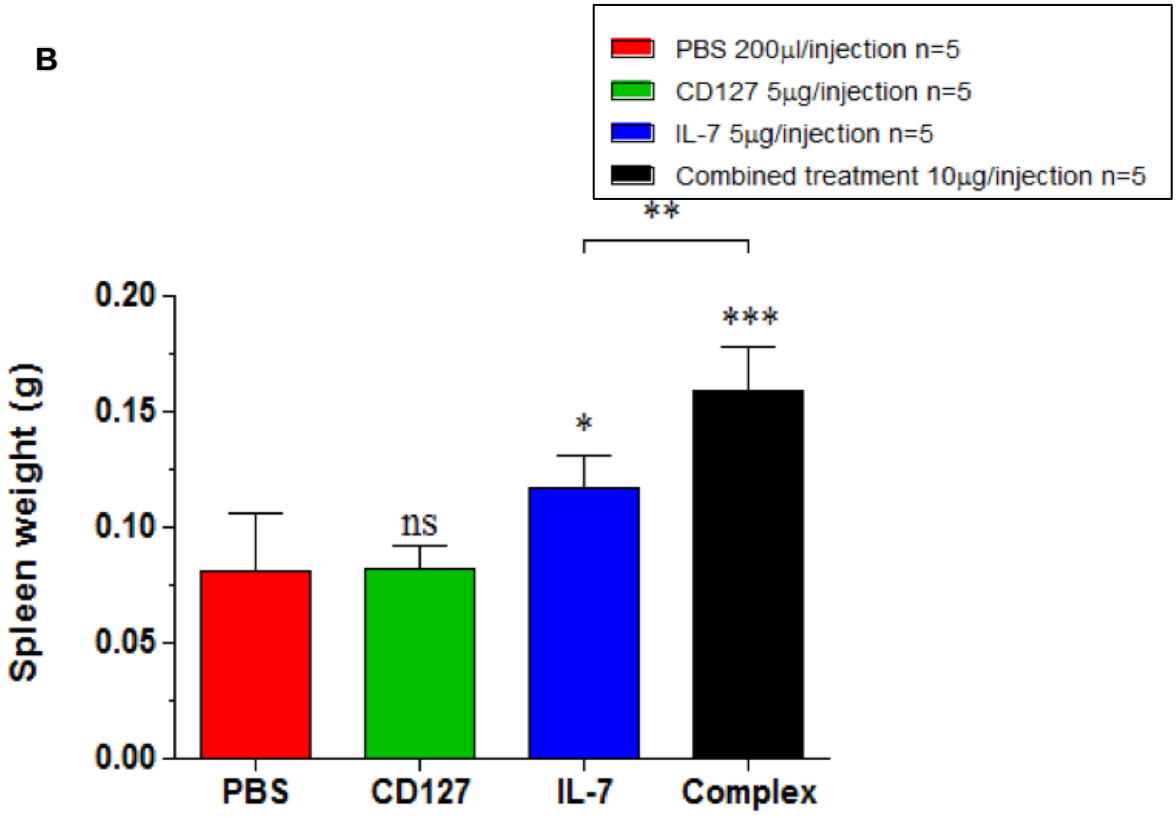
3.1.3.2 Combination of sCD127 and IL-7 enhances IL-7-mediated increase in $CD8^+$ T cells number, but not $CD4^+$ T cells in the spleen

With the positive impacts of both IL-7 and the combination therapies seen on T cell proliferation in peripheral lymphocytes and in the spleen, it was anticipated that there would be an increase in total lymphocyte numbers in the spleen of treated mice. To test this hypothesis, healthy mice were treated as indicated and the spleens were explanted at d5 to compare the treated spleens in terms of weight and total $CD4^+$ and $CD8^+$ T cell counts. The increase in the activity of T cell proliferation and cell number observed in peripheral blood and spleen correlated with a significant increase in spleen mass in mice treated either with IL-7 or combined treatment relative to control PBS treated mice (**Figure 3-6A** and **B)**. There was no significant difference in the total number of $CD4^+$ T cells in the spleen of treated mice when compared to PBS treated mice (**Figure 3-6C).** However, there was a significant increase in $CD8^+$ T cell count in the spleen of mice treated with IL-7 or combined therapy relative to mice treated with PBS (**Figure 3-6D)**.

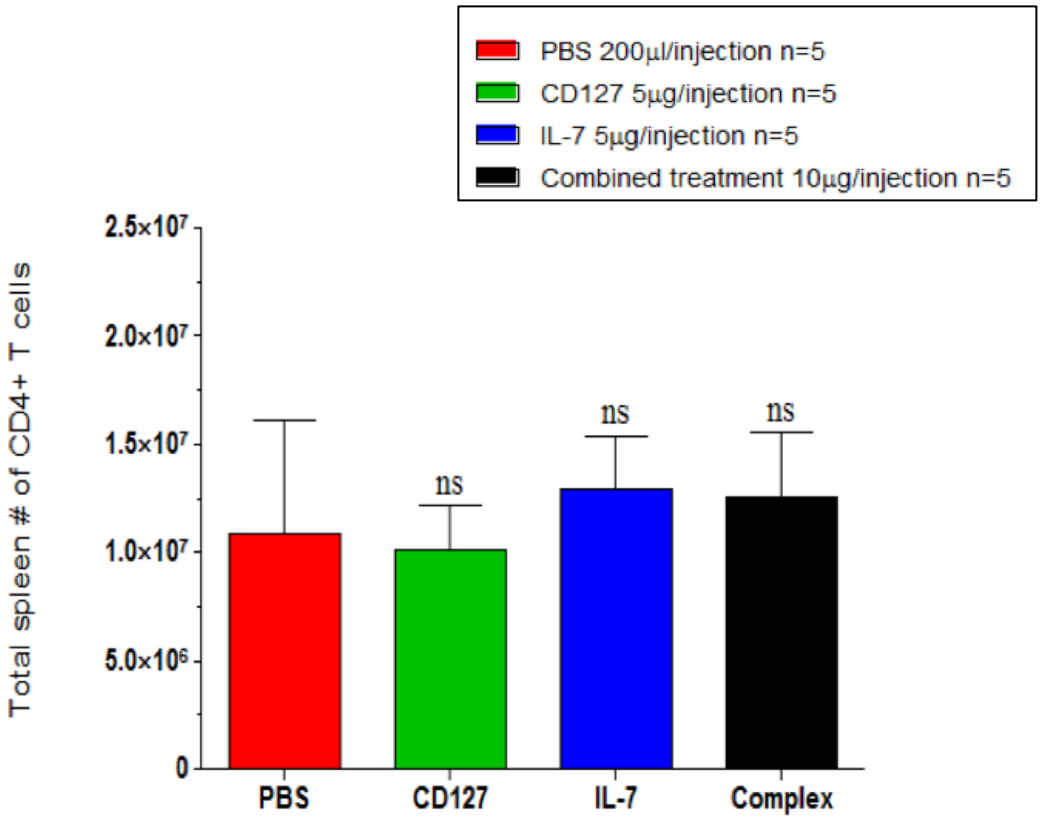
A


PBS
sCD127
IL-7
Complex

B


PBS 200µl/injection n=5
CD127 5µg/injection n=5
IL-7 5µg/injection n=5
Combined treatment 10µg/injection n=5
**
***
*
ns
Spleen weight (g)
0.20
0.15
0.10
0.05
0.00
PBS
CD127
IL-7
Complex

C


PBS 200µl/injection n=5
CD127 5µg/injection n=5
IL-7 5µg/injection n=5
Combined treatment 10µg/injection n=5
Total spleen # of CD4+ T cells
2.5x10⁷
2.0x10⁷
1.5x10⁷
1.0x10⁷
5.0x10⁶
0
ns
ns
ns
PBS
CD127
IL-7
Complex

D

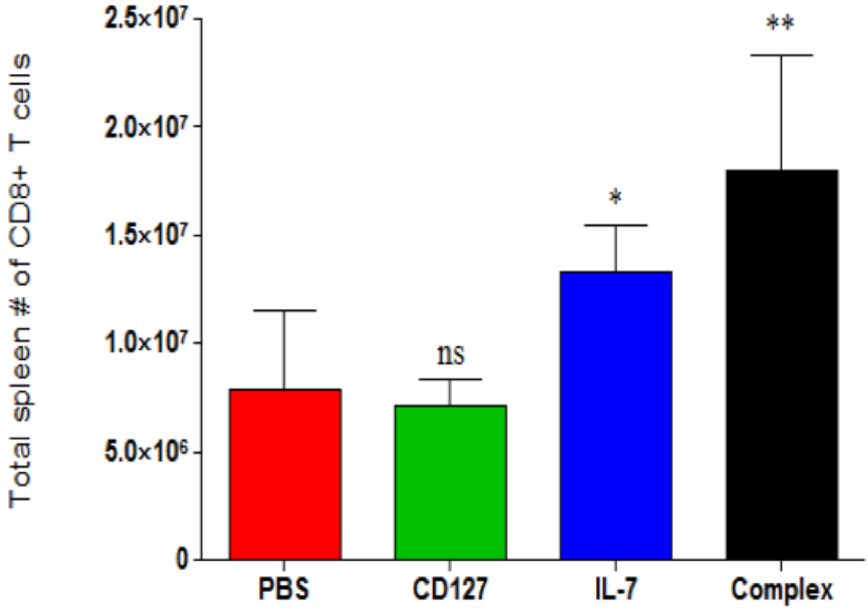
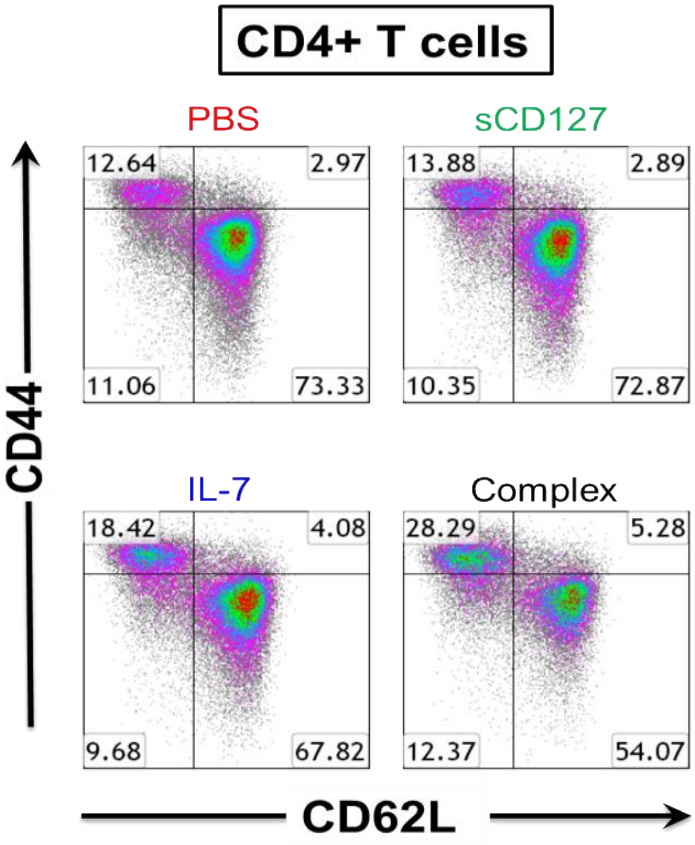

Total spleen # of CD8+ T cells
2.5x10⁷
2.0x10⁷
1.5x10⁷
1.0x10⁷
5.0x10⁶
0
ns
*
**
PBS
CD127
IL-7
Complex

Figure 3-6: The effect of IL-7 and sCD127 treatment on spleen T cell numbers in healthy C57BL/6 mice. Mice were divided into 4 groups and treated for 5 consecutive days (d0-d4) with either: 5 μg of IL-7, 5 μg of sCD127, a complex 5 μg of IL-7 and 5 μg of sCD127 (pre-incubated for 30 mins at 37°C) or PBS alone. On the day following the final i.p. injection, mice were sacrificed, and the spleens were harvested. **A)** Representative images of whole spleens from each treatment groups. **B)** Harvested spleens were weighed and the mean weights in grams per treatment group were reported ± SD. The total count of $CD4^+$ **(C)** and $CD8^+$ **(D)** T cells isolated from the spleens of mice in the four different groups were reported as mean ± SD. Statistical analysis was completed using a one-way ANOVA for more than two groups and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$), or significant with $*P<0.05$, $**P<0.01$, or $***P<0.001$.

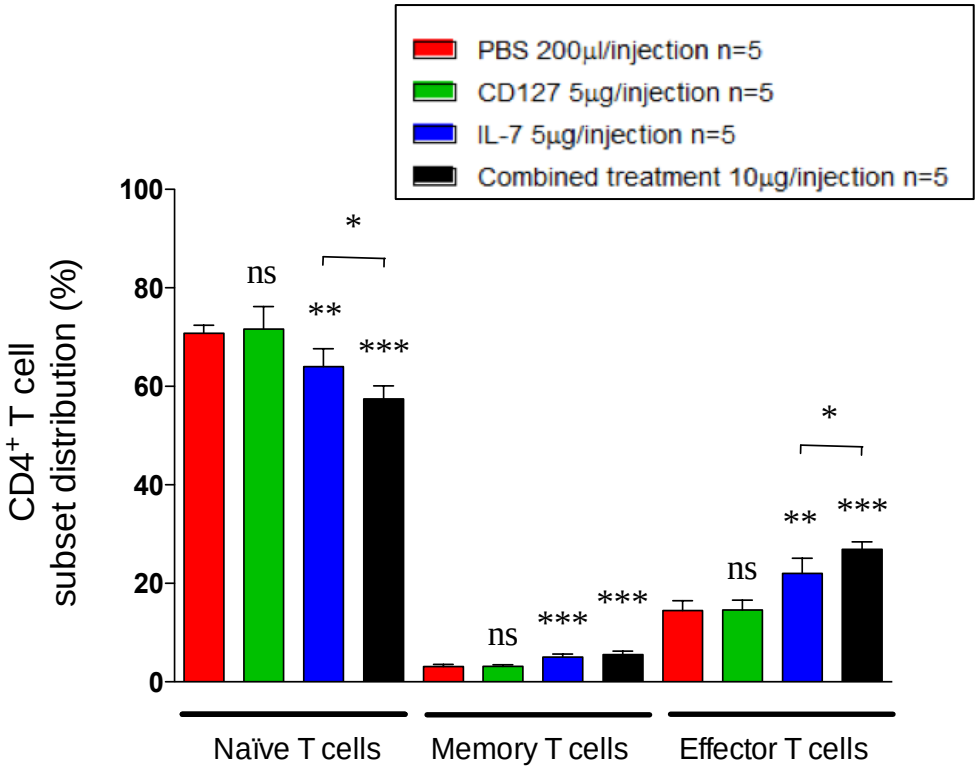
3.1.3.3 Treatment with sCD127 and IL-7 results in differentiation of naïve T cells into memory and effector T cell phenotypes

Next, we characterized the phenotype of $CD4^+$ and $CD8^+$ T cells generated following the indicated treatments. Naïve T cells (T_N), Memory T cells (T_M), and Effector T cells (T_E) populations were clearly distinguished by the use of two antibody combinations against homing receptor, CD62L, and CD44 antigen, and analyzed by flow cytometry. Naïve T cells were defined by $CD62L^{high}$ and $CD44^{low}$, memory T cells were known to highly express both surface markers, and effector T cells were defined by $CD62L^{low}$ and $CD44^{high}$.Interestingly, by d5 in both groups of mice treated with IL-7 and the combination therapy, upregulation of both CD62L and CD44 was observed in the $CD8^+$ T cell population, which resulted in a shift toward the T_M phenotype **Figure 3-7C and D**, whereas $CD4^+$ T cells acquire a more effector T cell-like phenotype **Figure 3-7A and B**. Of note, treating mice with complex therapy significantly enhanced the effect of IL-7 in mediating T cell differentiation relative to IL-7 therapy alone. However, there were no noticeable changes in the T cell phenotypes between mice treated with sCD127 alone and PBS (**Figure 3-7B and D**).

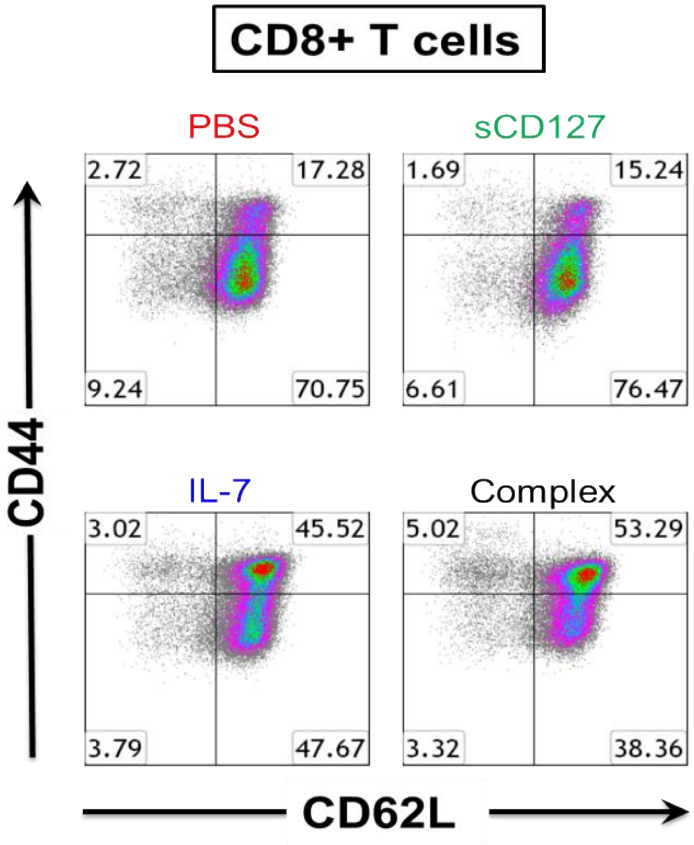
A


CD4+ T cells
PBS
sCD127
12.64
2.97
11.06
73.33
13.88
2.89
10.35
72.87
IL-7
Complex
18.42
4.08
9.68
67.82
28.29
5.28
12.37
54.07
CD44
CD62L

B


PBS 200µl/injection n=5
CD127 5µg/injection n=5
IL-7 5µg/injection n=5
Combined treatment 10µg/injection n=5
CD4+ T cell subset distribution (%)
100
80
60
40
20
0
ns
**
***
*
ns
***
***
ns
**
***
*
Naïve T cells
Memory T cells
Effector T cells

C


CD8+ T cells
PBS
sCD127
2.72
17.28
9.24
70.75
1.69
15.24
6.61
76.47
IL-7
Complex
3.02
45.52
3.79
47.67
5.02
53.29
3.32
38.36
CD44
CD62L

D

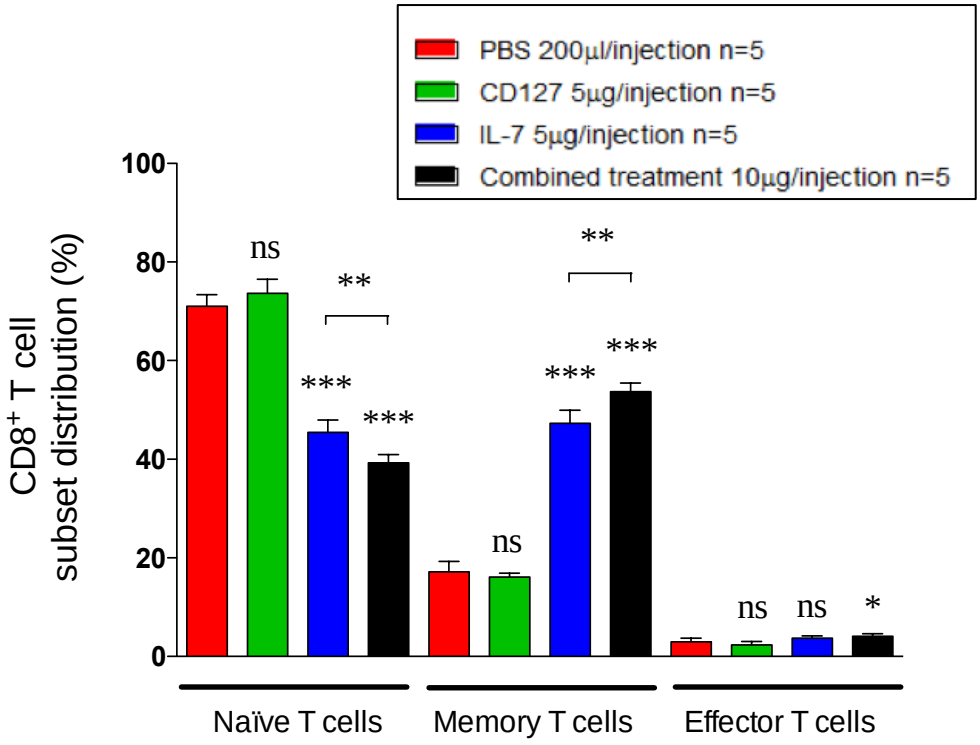

PBS 200µl/injection n=5
CD127 5µg/injection n=5
IL-7 5µg/injection n=5
Combined treatment 10µg/injection n=5
CD8+ T cell subset distribution (%)
100
80
60
40
20
0
ns
**
***
***
**
***
***
ns
ns
ns
*
Naïve T cells
Memory T cells
Effector T cells

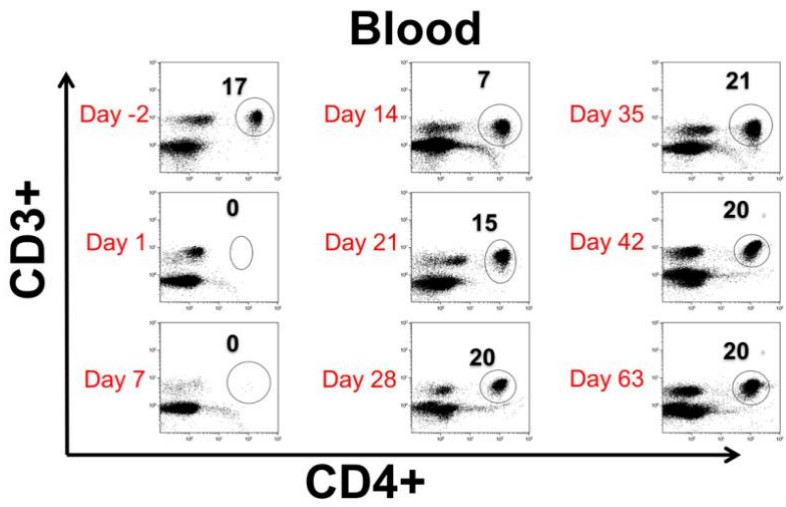
Figure 3-7: The effect of sCD127 and IL-7 treatment on spleen T cell subsets in healthy C57BL/6 mice. Mice were divided into 4 groups and treated for 5 consecutive days (d0-d4) with either: 5 μg of IL-7, 5 μg of sCD127, a complex 5 μg of IL-7 and 5 μg of sCD127 (pre-incubated for 30 mins at 37°C) or PBS alone. On the day following the final i.p. injection (d5), mice were sacrificed and the spleens were harvested. **A)** Representative scatter plots gated on $CD4^+$ T cells showing their expression levels of CD44 and CD62L for the four different treatment groups. **B)** Quantification of $CD4^+$ T cells isolated from the spleen expressing either naïve T cell (CD44 low, CD62L high); central memory T cell (CD44 high, CD62L high); or effector T cell (CD44 high, CD62L low) markers. **C)** Representative scatter plots gated on $CD8^+$ T cells showing expression levels of CD44 and CD62L for the four different treatment groups. **D)** Quantification of $CD8^+$ T cells isolated from the spleen expressing either naïve T cell, central memory T cell, or effector T cell markers. In B and D data represent mean ± SD. Statistical analysis was completed using one-way ANOVA for more than two groups and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$), or significant with *$P<0.05$, **$P<0.01$, or ***$P<0.001$.

3.2 Kinetics of $CD4^+$ and $CD8^+$ T cell reconstitution in antibody-mediated T cell depletion model, and the effect on endogenous IL-7 levels

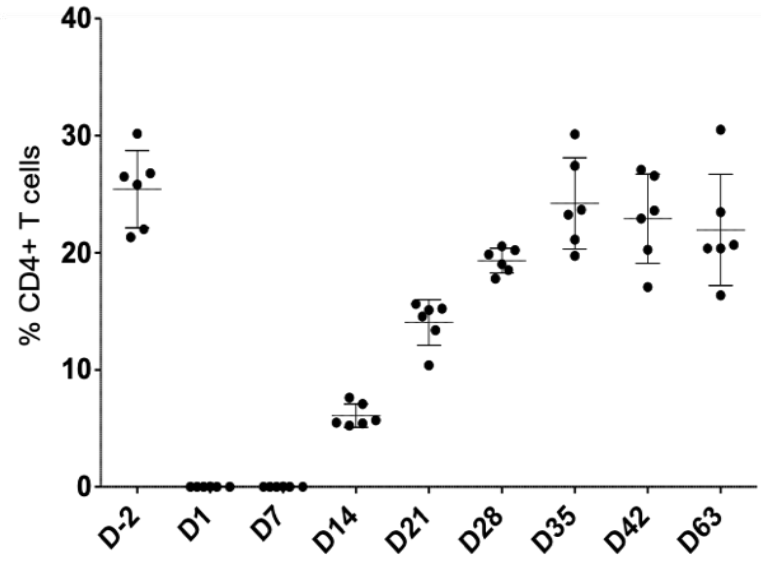
3.2.1 Following T cell depletion, complete reconstitution was observed in $CD4^+$ T cells relative to the partial reconstitution in $CD8^+$ T cells

In order to optimize and characterize a model of T cell depletion, blood samples were collected from six female WT C57BL/6 mice via their saphenous vein as mentioned before (**See Figure 2-2**). PBMCs were isolated, and stained with antibodies against CD3, CD4, and CD8 to characterize the population of $CD4^+$ and $CD8^+$ T cells by flow cytometry (baseline level). Next, the mice were injected i.p. with 150 µg of anti-CD4 and anti-CD8 depleting antibodies. We observed that both T cell subsets rapidly and completely depleted from mice blood one day following the antibody administration (**Figure 3-8**). Mice were then bled weekly to demonstrate the kinetics of T cell reconstitution following depletion (from d7 to d63). Our result revealed that the pattern of T cell reconstitution was different between the T cell types; an almost complete T cell reconstitution was observed in $CD4^+$ T cells 6 weeks following depletion **Figure 3-8A, B, and C**, while $CD8^+$ T cells remained partially reconstituted **Figure 3-8D, E, and F**. In addition, plasma was collected for measurement of IL-7 by cytometric bead array throughout the timeline of the experiment. IL-7 was not detectable in the plasma of depleted mice at any time points that we assessed **Figure 3-9**.

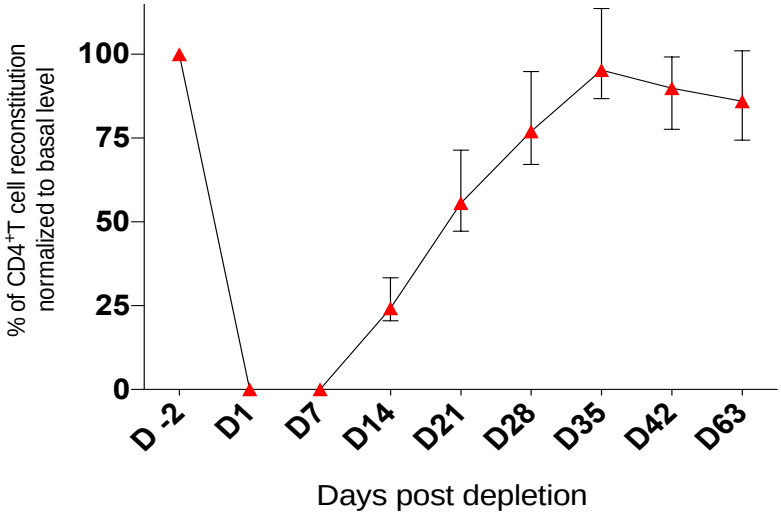
A


Blood
CD3+
CD4+
Day -2
17
Day 14
7
Day 35
21
Day 1
0
Day 21
15
Day 42
20
Day 7
0
Day 28
20
Day 63
20

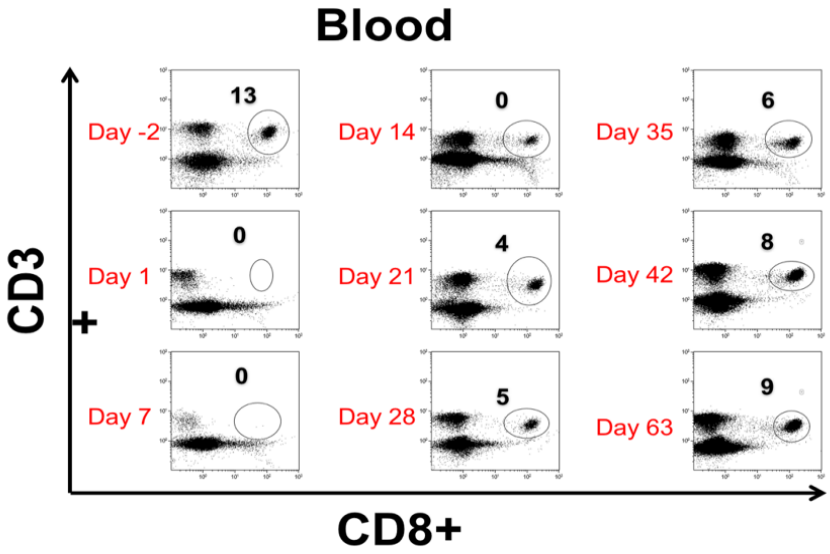
B


% CD4+ T cells
40
30
20
10
0
D-2
D1
D7
D14
D21
D28
D35
D42
D63

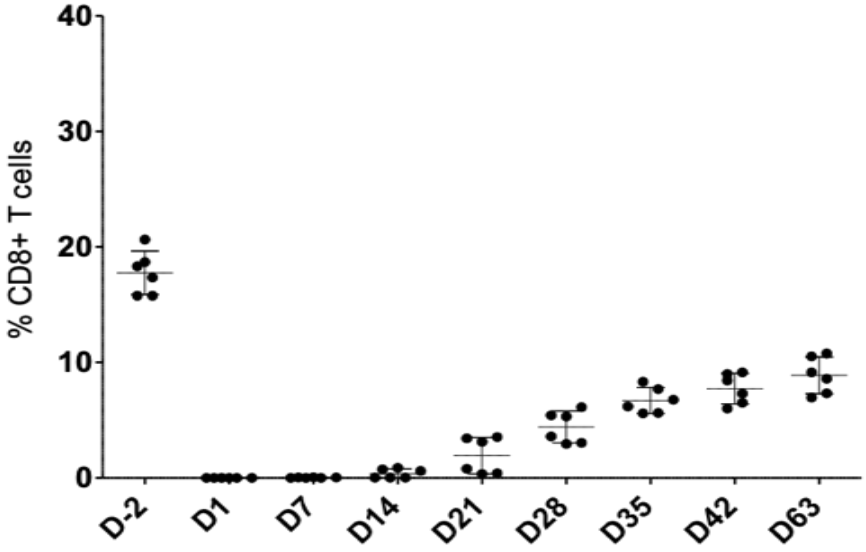
C


% of CD4+T cell reconstitution normalized to basal level
100
75
50
25
0
D -2
D1
D7
D14
D21
D28
D35
D42
D63
Days post depletion

D


Blood
Day -2
13
Day 14
0
Day 35
6
Day 1
0
Day 21
4
Day 42
8
Day 7
0
Day 28
5
Day 63
9
CD3
+
CD8+

E


% CD8+ T cells
40
30
20
10
0
D-2
D1
D7
D14
D21
D28
D35
D42
D63

F

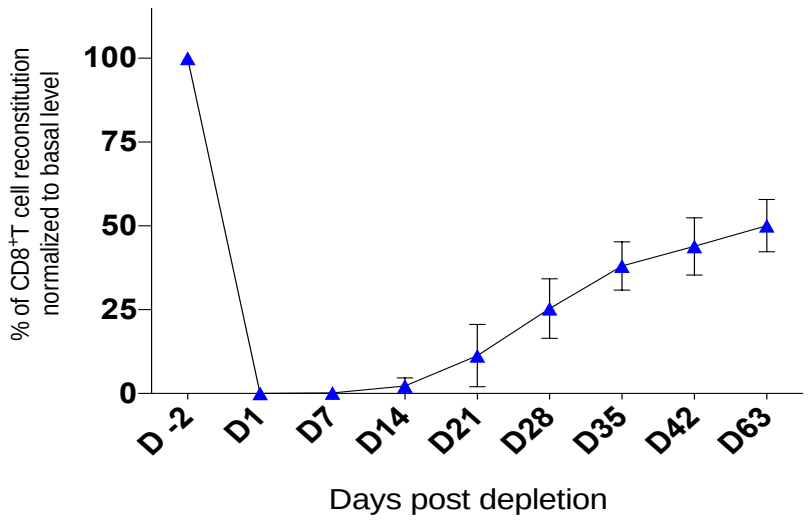

% of CD8+T cell reconstitution normalized to basal level
100
75
50
25
0
D -2
D1
D7
D14
D21
D28
D35
D42
D63
Days post depletion

Figure 3-8: The kinetics of T cell reconstitution following T cell depletion in C57BL/6 mice. n=6. Mice were treated with a single i.p. injection of 150 μg of both anti-CD4 and anti-CD8 antibodies. Representative dot plots show the kinetics of T cell reconstitution for $CD4^+$ T cells **(A)** and $CD8^+$ T cells **(D)** in a single mouse. The frequency of $CD4^+$ T cells **(B)** and $CD8^+$ T cells **(E)** in the blood before antibody treatment (d-2), immediately after antibody treatment (d1) and as the T cell slowly start to be reconstituted up to d63. The % of $CD4^+$ T cells or $CD8^+$ T cells reconstituted over time normalized to the basal level are shown in graphs **C** ($CD4^+$ T cells) and **F** ($CD8^+$ T cells).

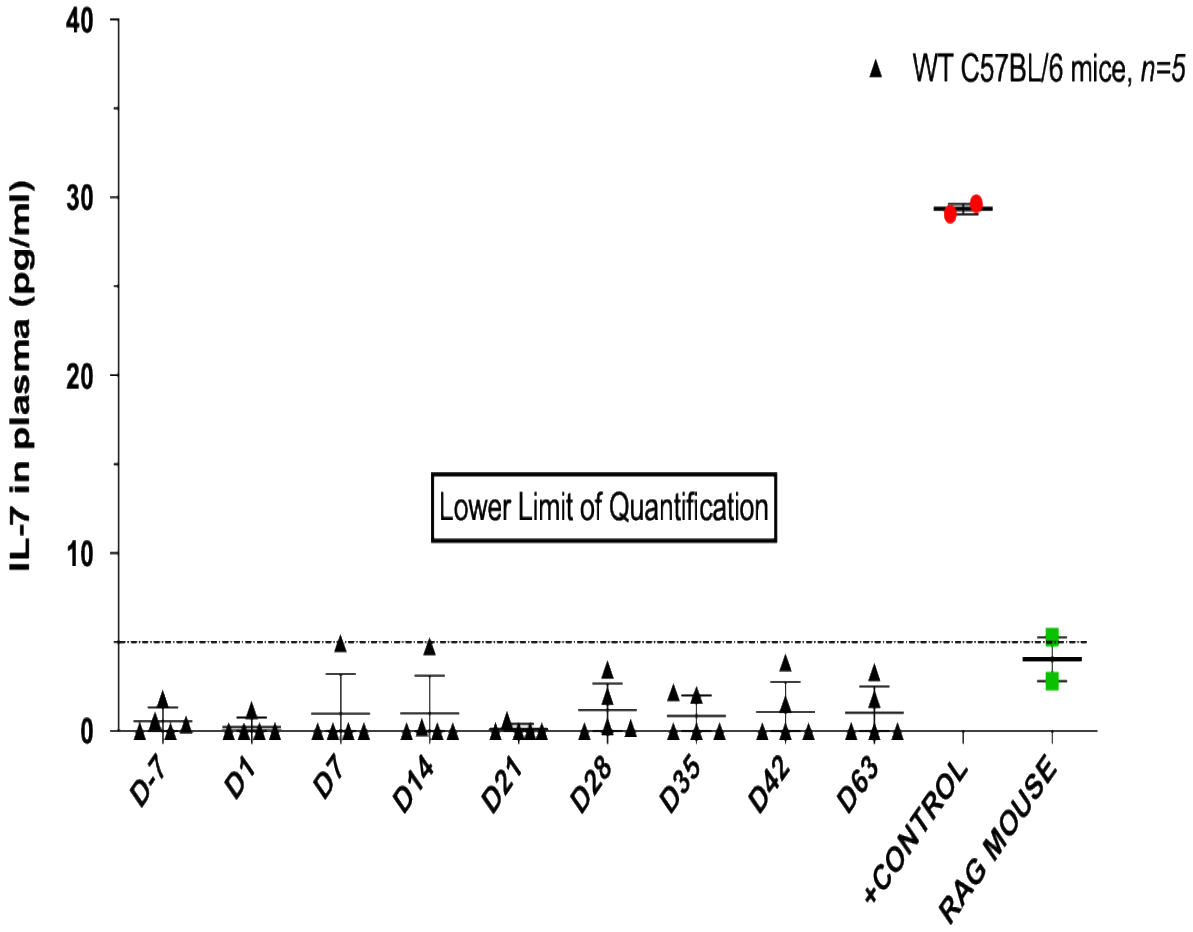
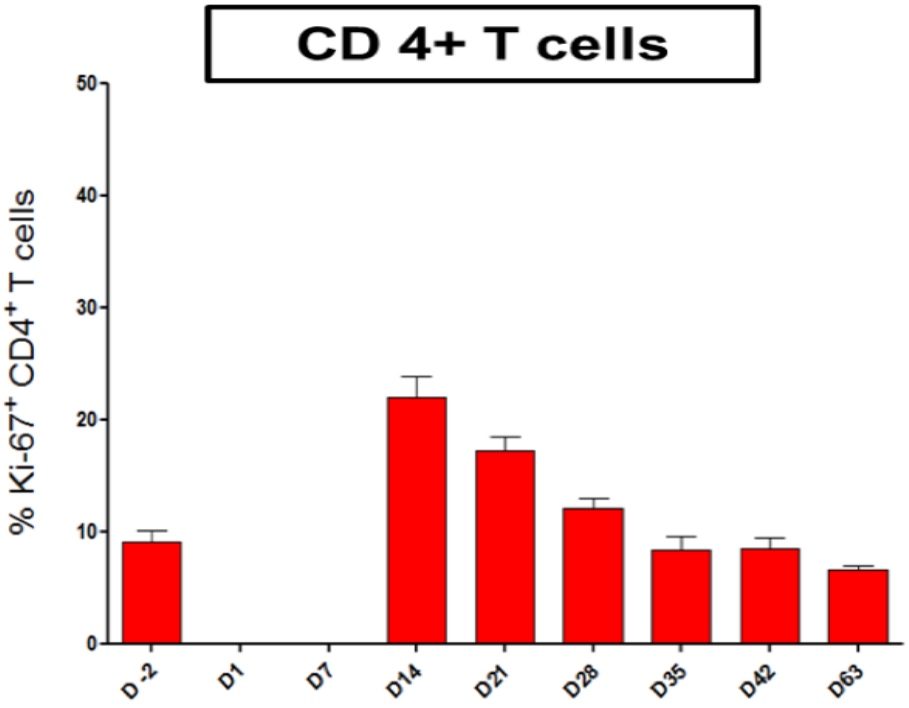

40
30
20
10
0
IL-7 in plasma (pg/ml)
WT C57BL/6 mice, n=5
Lower Limit of Quantification
D-7
D1
D7
D14
D21
D28
D35
D42
D63
+CONTROL
RAG MOUSE

Figure 3-9 Plasma concentration of circulating IL-7 in T-cell depleted mice, n=5. Mice were treated with a single i.p. injection of 150 μg of both anti-CD4 and anti-CD8 antibodies. Quantification of IL-7 in mice plasma was performed by CBA and samples were collected before antibody treatment (d-7), immediately after antibody treatment (d1) and weekly up to d63. For controls, IL-7 standard antigen was used as a positive control, and plasma from RAG Knockout mice was used as an experimental control.

3.2.2 Ki-67 levels with T cell depletion

Next, we assessed the kinetics of Ki-67 expression following T cell depletion in both $CD4^+$ and $CD8^+$ T cells. The pattern of the proliferation profile was consistent between the T cell subtypes, in which expression of Ki-67 was found to be at the highest level when T cells were mostly depleted at d14 and d21 for $CD4^+$ and $CD8^+$ T cells respectively. This increase in proliferation rate returned to the basal level after 6 weeks of depletion (d42), indicating a termination of an active cell division **Figure 3-10A and B**.

A


CD 4+ T cells
% Ki-67+ CD4+ T cells
0
10
20
30
40
50
D -2
D1
D7
D14
D21
D28
D35
D42
D63

B

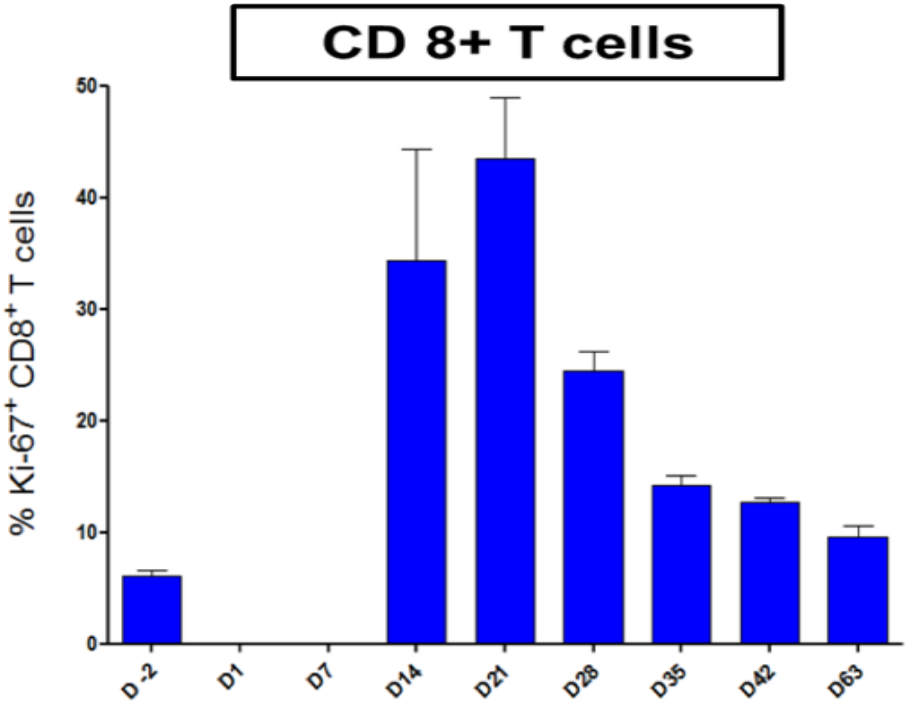
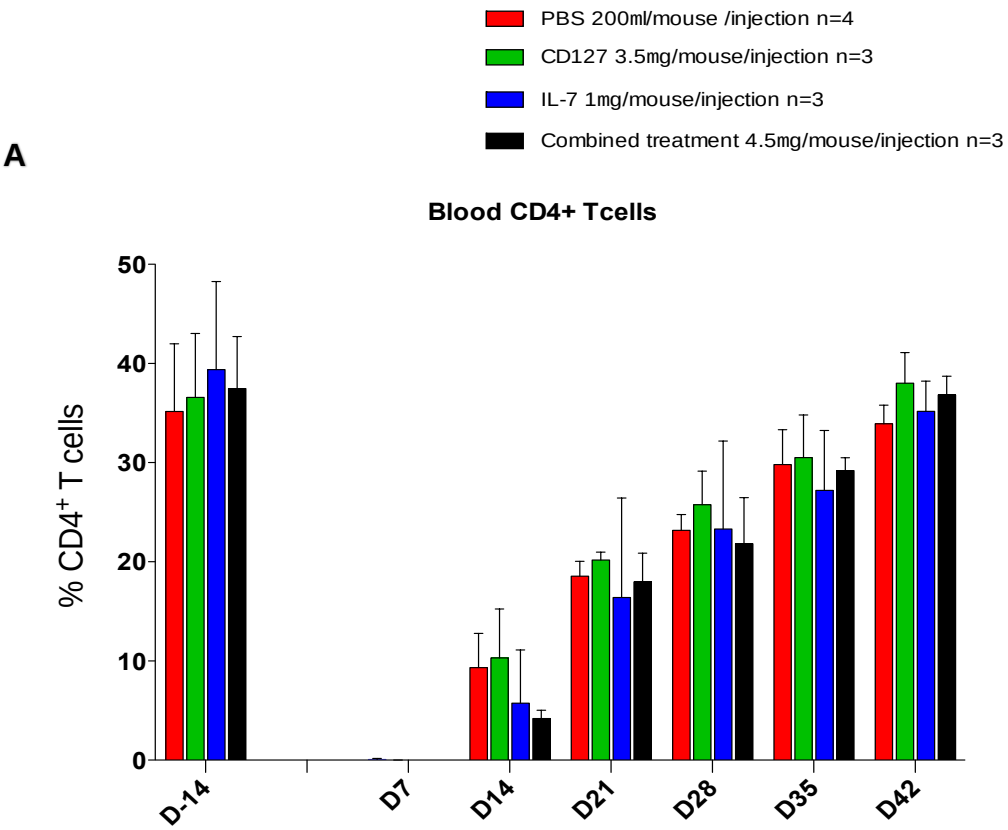

CD 8+ T cells
% Ki-67+ CD8+ T cells
0
10
20
30
40
50
D -2
D1
D7
D14
D21
D28
D35
D42
D63

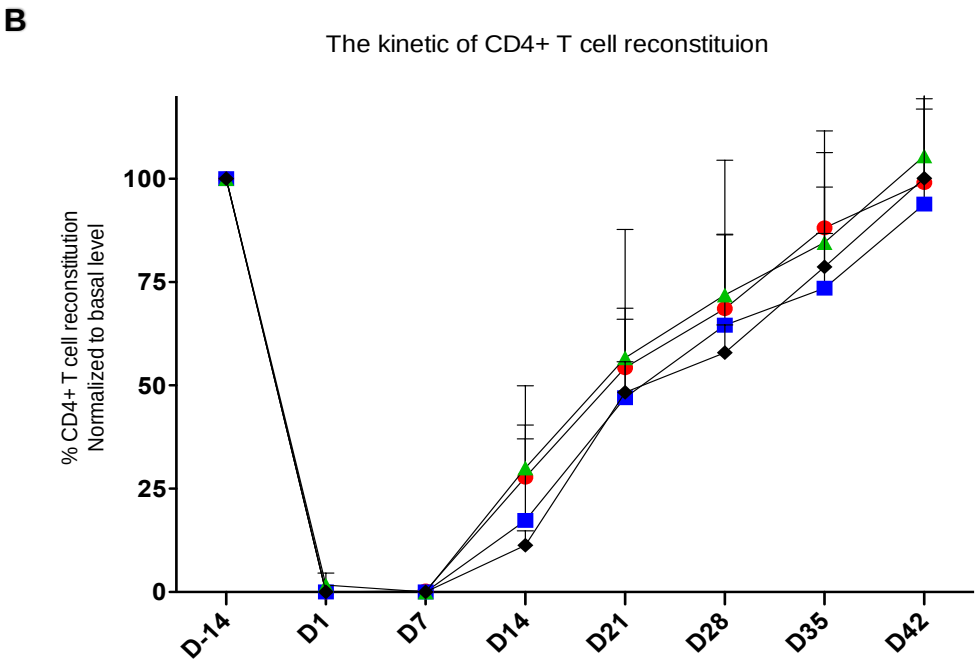
Figure 3-10: The kinetics of T cell reconstitution following T cell depletion in C57BL/6 mice as measured by T cells expressing Ki-67. n=6. The frequency of $CD4^+$ T cells **(A)** and $CD8^+$ T cells **(B)** expressing Ki-67 was quantified in blood cells from mice pre-treatment and from mice treated with anti-CD4 and anti-CD8 antibodies (150 μg) for up to d63.

3.3 The effect of sCD127 treatment with or without IL-7 on T cell reconstitution and proliferation in the T cell depletion model

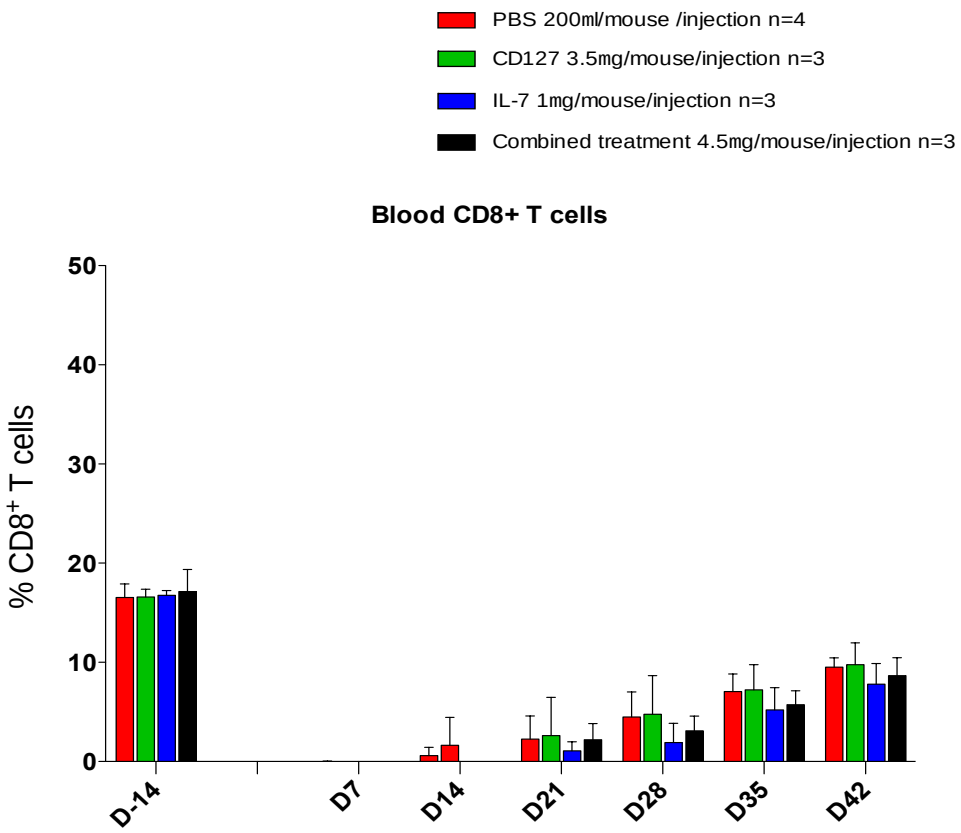
3.3.1 $CD8^+$ T cell frequencies but not $CD4^+$ T cells are increased in response to IL-7 and combined therapy,

After optimizing the T cell depletion model, we found that at d42 most of the $CD4^+$ T cells were reconstituted, whereas $CD8^+$ T cells were only partially reconstituted, and remained to at later time points. Therefore, d42 was chosen to be the endpoint for the subsequent T cell depletion experiments. Next, we investigated whether treatment with IL-7 and/or sCD127 would enhance the reconstitution of T cells following T cell depletion. At first, a total of 13 mice were divided into the four groups (**See Figure 2-3A**). Our preliminary data suggested that two doses administered at d6 and d13 had no significant impact on T cell reconstitution in peripheral blood compared to PBS control group **Figure 3-11A-D**. Therefore, we modified the dosage time and concentration, and assessed T cell reconstitution. This time, mice were treated for 5 consecutive days (d24-d28) (**See Figure 2-3B**). The results revealed that treatment with IL-7 or combined therapy had minimal impact on the distribution of $CD4^+$ T cells in the blood **Figure 3-11E and F**, while a significantly increase in the $CD4^+$ T cell number was observed **Figure 3-11I**. In contrast, the $CD8^+$ T cell proportion and the total $CD8^+$ T cell number in the blood were enhanced at d29 when mice were treated with IL-7 or combined therapy relative to PBS **Figure 3-11G, H, and J**. However, This only reached statistical significant in the total cell count analysis **Figure 3-11J**. Interestingly, the enhanced T cell reconstitution was transient as seen at d29 (mice were treated until d28) and disappeared later after treatment discontinuation. Of note, there was no significant difference in the effects between IL-7 and IL-7 complexed with sCD127 on T cell reconstitution (**Figure 3-11**).

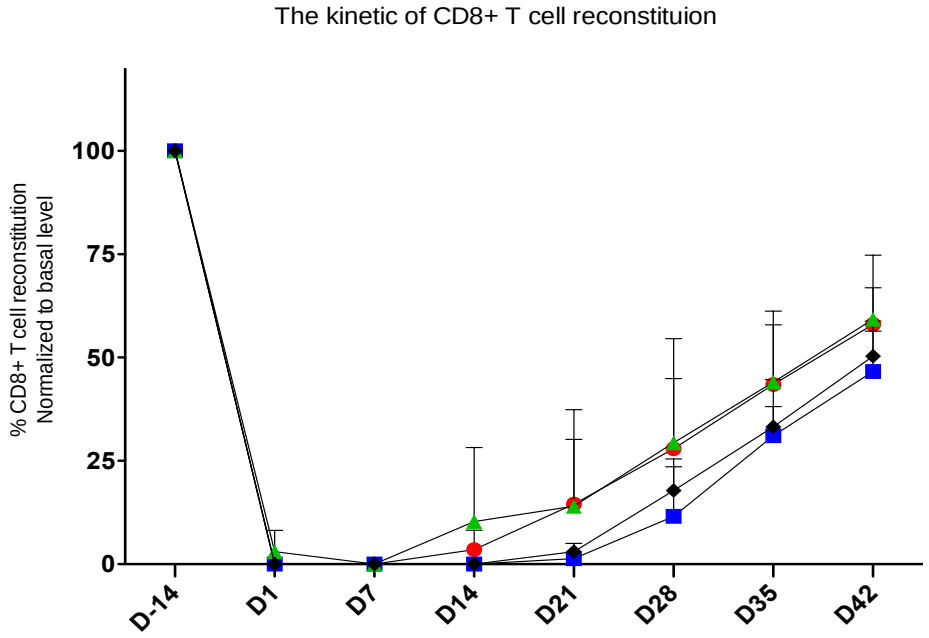

A
PBS 200ml/mouse /injection n=4
CD127 3.5mg/mouse/injection n=3
IL-7 1mg/mouse/injection n=3
Combined treatment 4.5mg/mouse/injection n=3
Blood CD4+ Tcells
% CD4+ T cells
50
40
30
20
10
0
D-14
D7
D14
D21
D28
D35
D42

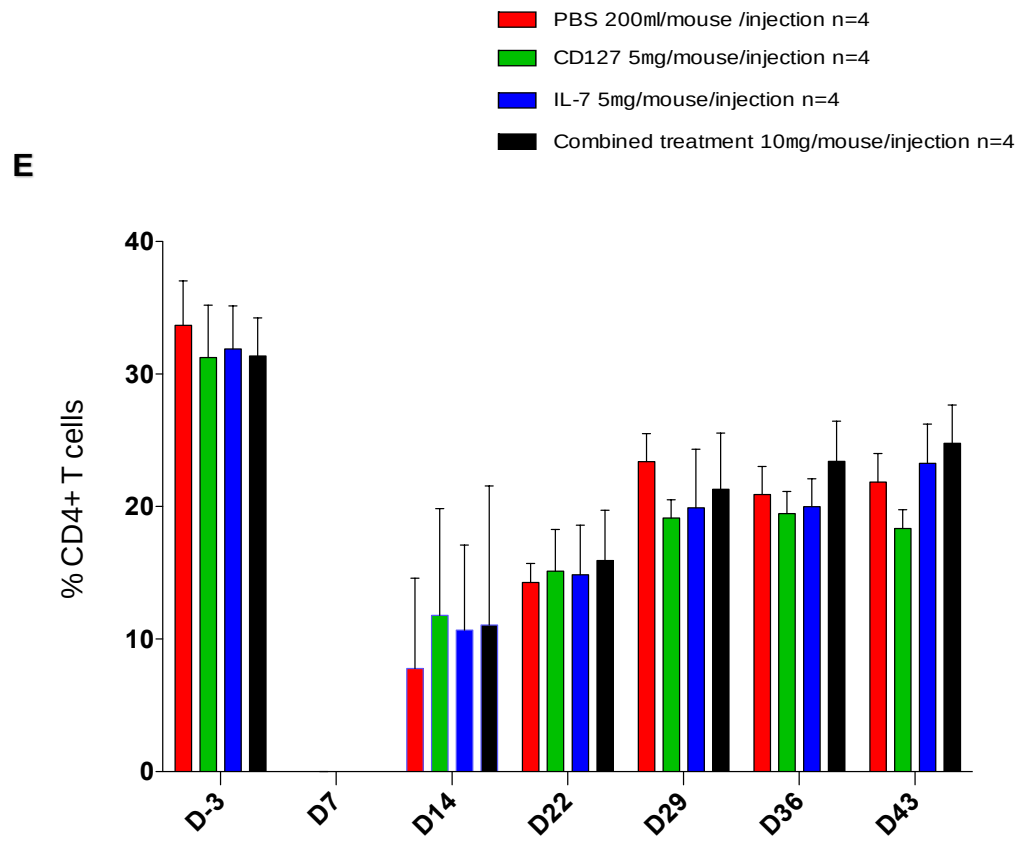

B
The kinetic of CD4+ T cell reconstituion
% CD4+ T cell reconstitution
Normalized to basal level
100
75
50
25
0
D-14
D1
D7
D14
D21
D28
D35
D42

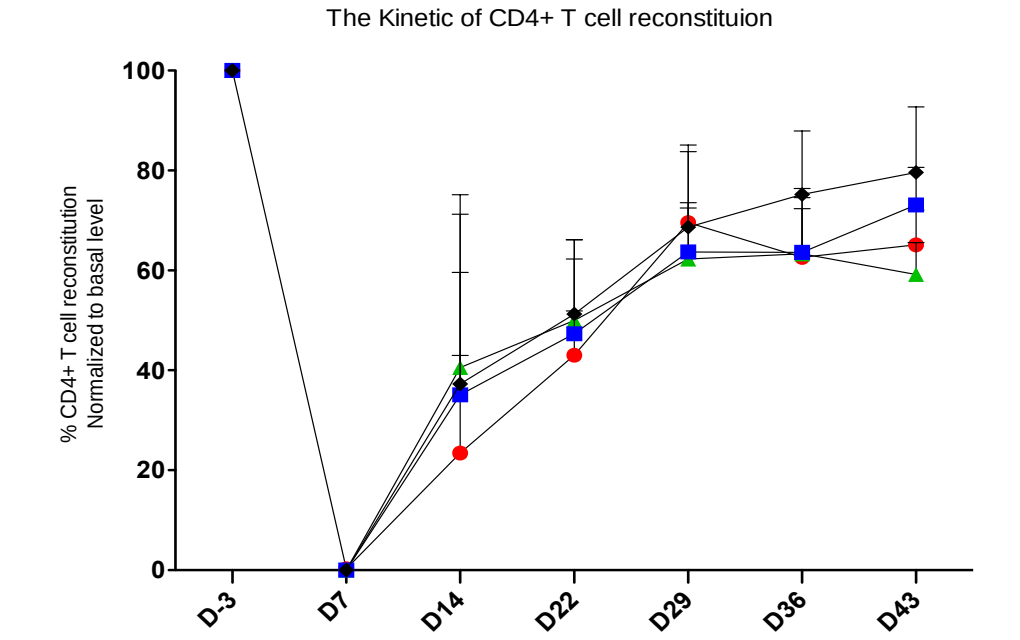
C


PBS 200ml/mouse /injection n=4
CD127 3.5mg/mouse/injection n=3
IL-7 1mg/mouse/injection n=3
Combined treatment 4.5mg/mouse/injection n=3
Blood CD8+ T cells
% CD8+ T cells
50
40
30
20
10
0
D-14
D7
D14
D21
D28
D35
D42

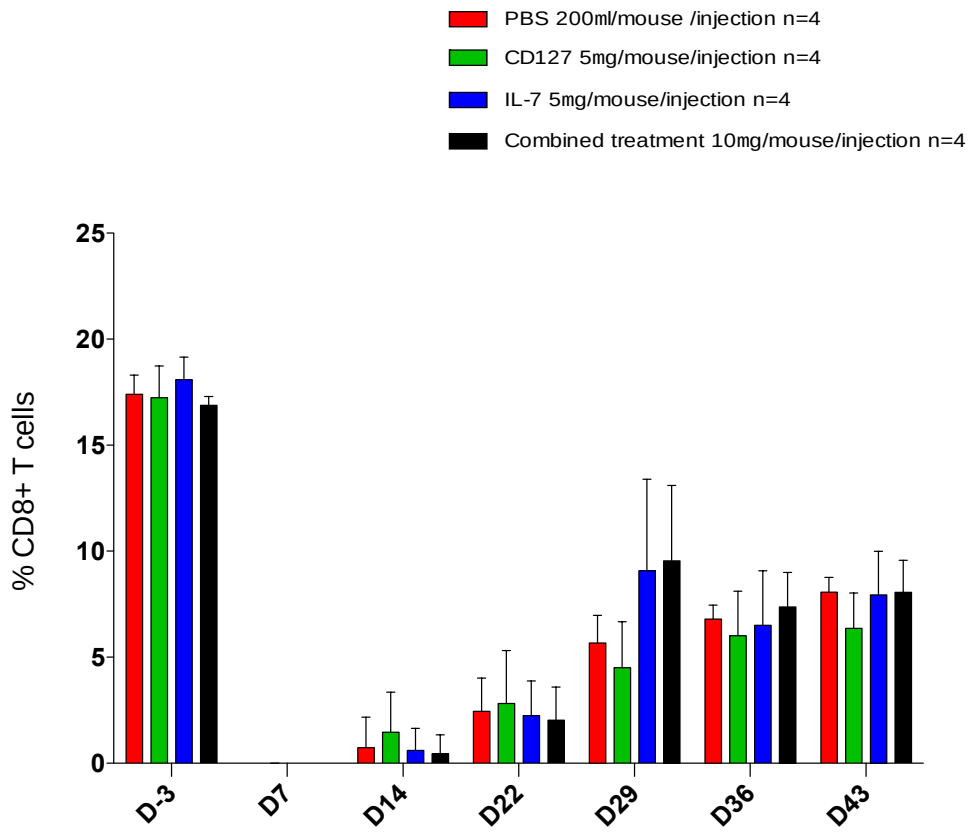
D


The kinetic of CD8+ T cell reconstituion
% CD8+ T cell reconstitution
Normalized to basal level
100
75
50
25
0
D-14
D1
D7
D14
D21
D28
D35
D42

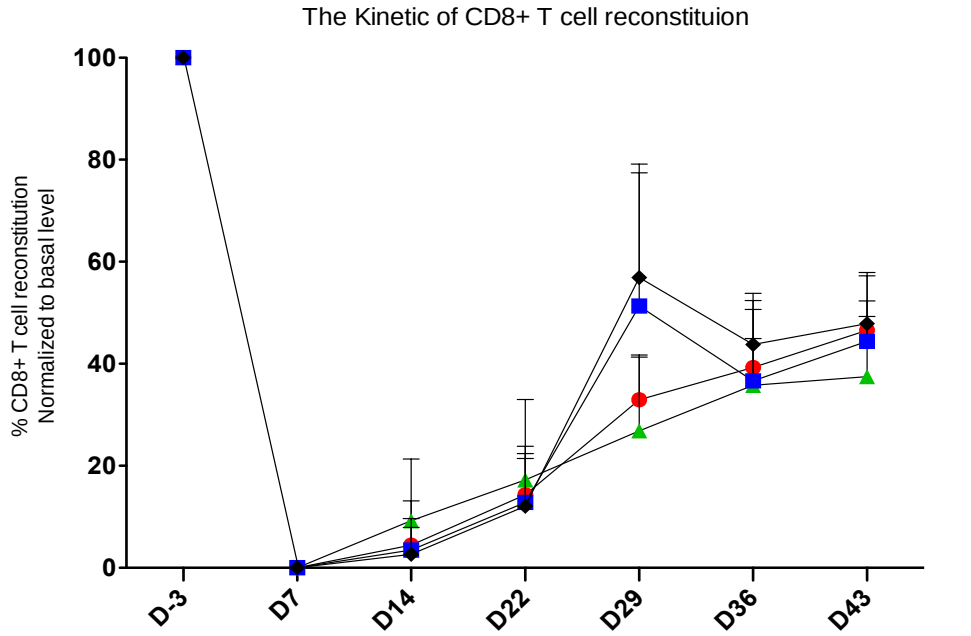

PBS 200ml/mouse /injection n=4
CD127 5mg/mouse/injection n=4
IL-7 5mg/mouse/injection n=4
Combined treatment 10mg/mouse/injection n=4
E
% CD4+ T cells
40
30
20
10
0
D-3
D7
D14
D22
D29
D36
D43


F
The Kinetic of CD4+ T cell reconstituion
% CD4+ T cell reconstitution
Normalized to basal level
100
80
60
40
20
0
D-3
D7
D14
D22
D29
D36
D43

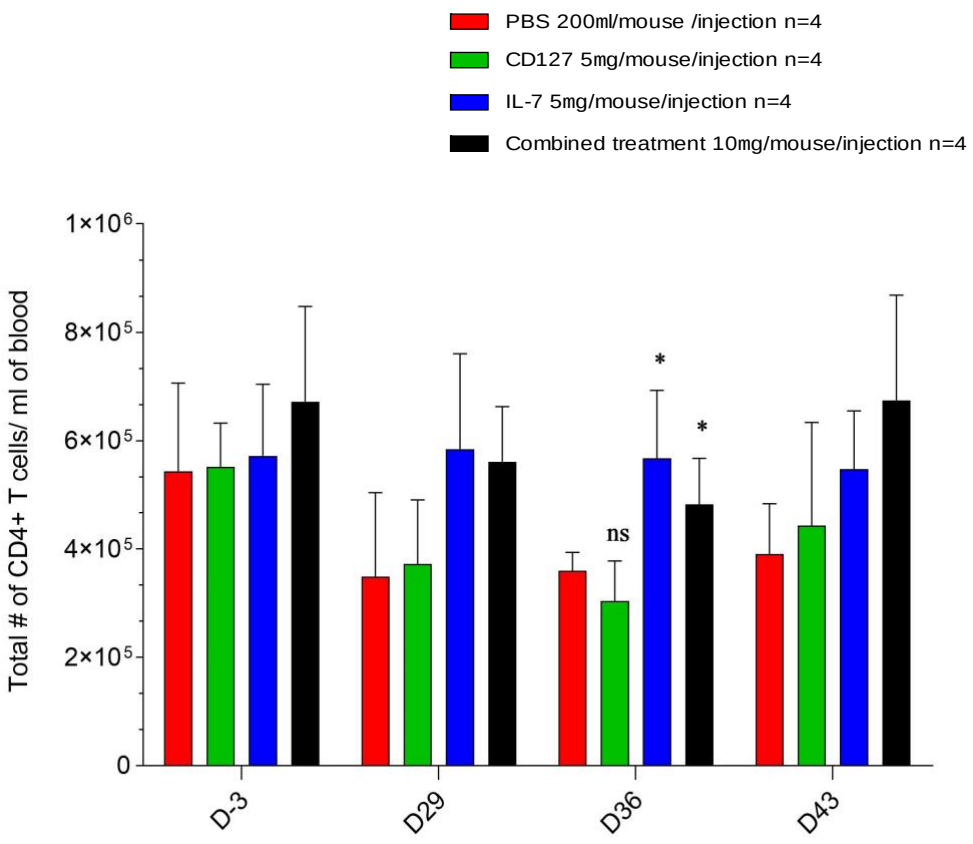
G


PBS 200ml/mouse /injection n=4
CD127 5mg/mouse/injection n=4
IL-7 5mg/mouse/injection n=4
Combined treatment 10mg/mouse/injection n=4
% CD8+ T cells
25
20
15
10
5
0
D-3
D7
D14
D22
D29
D36
D43

H


The Kinetic of CD8+ T cell reconstituion
% CD8+ T cell reconstitution
Normalized to basal level
100
80
60
40
20
0
D-3
D7
D14
D22
D29
D36
D43

I


PBS 200ml/mouse /injection n=4
CD127 5mg/mouse/injection n=4
IL-7 5mg/mouse/injection n=4
Combined treatment 10mg/mouse/injection n=4
Total # of CD4+ T cells/ ml of blood
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
ns
*
*
D-3
D29
D36
D43

J

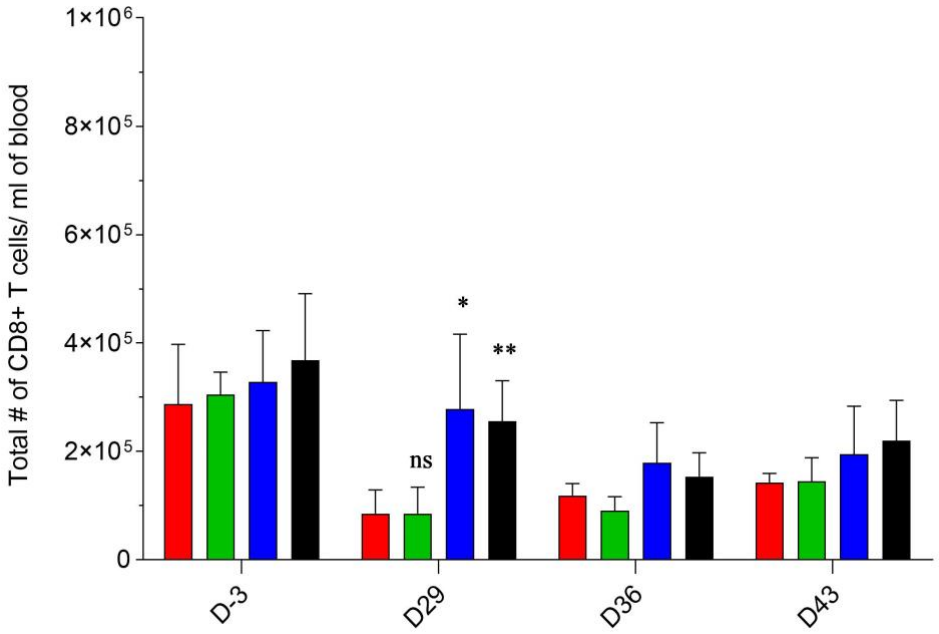
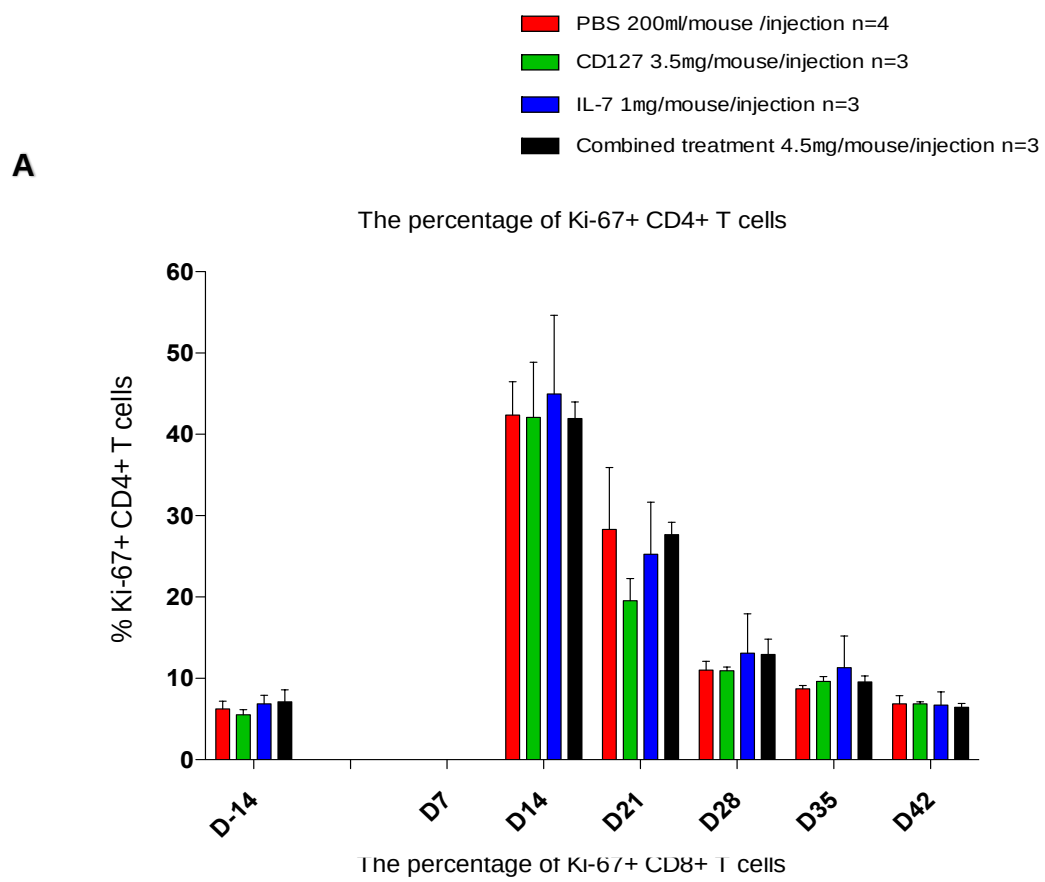

Total # of CD8+ T cells/ ml of blood
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
ns
*
**
D-3
D29
D36
D43

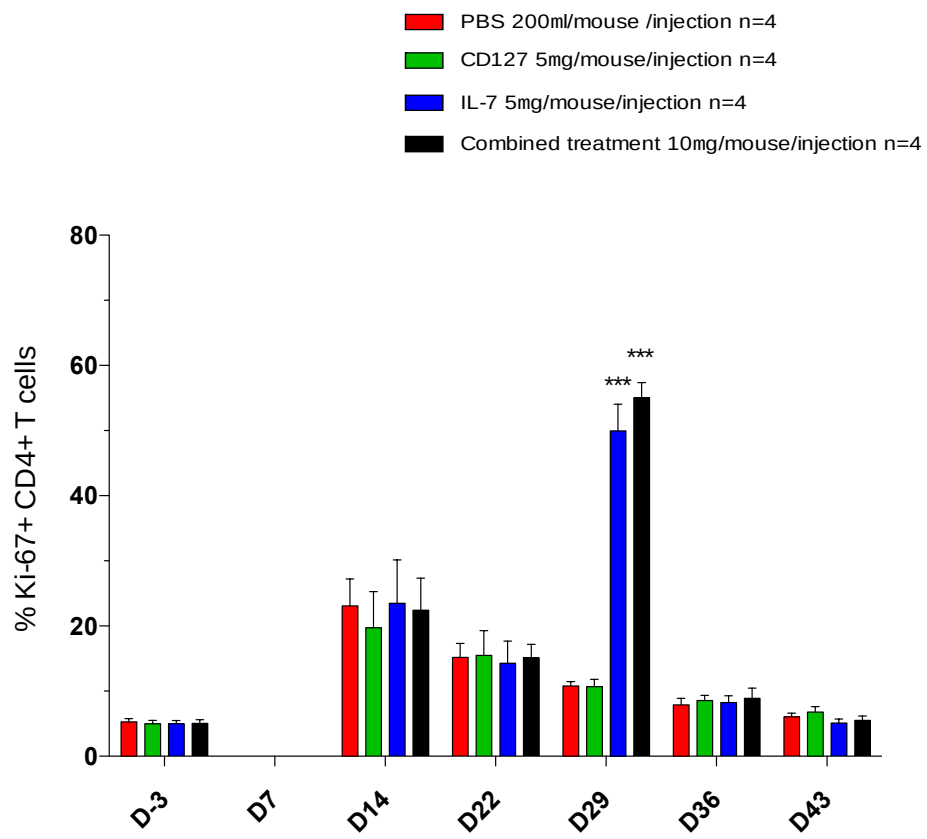
Figure 3-11: The effect of sCD127 treatment on blood T cell reconstitution in T cell depleted WT C57BL/6 mice. For T cell depletion, mice were injected with one dose of anti-CD4 and anti-CD8 depletion antibodies (150μg for each antibody). **A-D)** The effect of low, two doses sCD127 and/or IL-7 on T cell reconstitution in the blood of depleted C57BL/6 mice. Mice were divided into 4 groups, and treated twice with either 1 μg of IL-7, 3.5 μg of sCD127, 4.5 μg of IL-7 and sCD127 complexed (pre-incubated together for 30 mins at 37°C), or 200 μl PBS alone (vehicle control). The graphs show the frequency of $CD4^+$ T cells **(A)** and $CD8^+$ T cells **(C)** in the blood before antibody treatment (d-14), immediately after antibody treatment (d1), and as the T cell slowly start to be reconstituted up to d42. The % of $CD4^+$ T cells or $CD8^+$ T cells reconstituted over time normalized to the basal level are shown in graphs **B** ($CD4^+$ T cells) and **D** ($CD8^+$ T cells). N=3 mice per treatment groups and n=4 mice per PBS group. **E-J)** The effect of five consecutive days of high dose sCD127 and/or IL-7 on T cell reconstitution in the blood of depleted C57BL/6 mice. Mice were divided into 4 groups, n= 4 mice per group, and treated by i.p. injection for five consecutive days (between d24 – 28) with either: 5 μg of IL-7, 5 μg of sCD127, 10 μg of both treatments complexed (pre-incubated for 30 mins at 37°C), or 200 μl of PBS alone (vehicle control). The graphs show the frequency of $CD4^+$ T cells **(E)** and $CD8^+$ T cells **(G)** in the blood before antibody treatment (d-3), and as the T cell slowly start to be reconstituted up to d42. The % of $CD4^+$ T cells or $CD8^+$ T cells reconstituted over time normalized to the basal level are shown in graphs **F** ($CD4^+$ T cells) and **H** ($CD8^+$ T cells). Total blood count of $CD4^+$ **(I)** and $CD8^+$ T cells **(J)**. Data represent mean ± SD. Statistical analysis was completed using one-way ANOVA for more than two groups and a Student's two-tailed unpaired t-test was used for comparisons between two groups. P values are listed as either not significant (ns, $P>0.05$), or significant with *$P<0.05$, **$P<0.01$, or ***$P<0.001$.

3.3.2 Both IL-7 and combined treatment robustly and transiently enhance T cell proliferation

Next, we assessed the effect of IL-7 and/or sCD127 treatments on the kinetics of Ki-67 expression following T cell depletion in both $CD4^+$ and $CD8^+$ T cells. Our initial investigation of the first approach as described in **Figure 2-3**A demonstrated there was no difference in the overall expression of Ki-67 in either $CD4^+$ or $CD8^+$ T cells between PBS treated mice or any other treatments at the indicated time points **Figure 3-12A and B.** This was consistent with the lack of impact on T cell reconstitution. However, after increasing the dosage time and concentration of the treatments we found that there was a robust increase in the expression of Ki-67 within both cell types at d29 when mice were treated with IL-7 or combined therapy. Approximately 50% of $CD4^+$ T cells and 85% of $CD8^+$ T cells were Ki-67+ relative in mice treated with IL-7 alone compared to 10% and 26%, respectively in PBS-only treated mice **Figure 3-12C and D**. Mice treated with combined therapy showed a greater response than IL-7 in which approximately 55% of $CD4^+$ T cells and 88% of $CD8^+$ T cells were Ki-67+. Of note, the difference in Ki-67 expression was only statistically significant between mice treated with IL-7 and combined therapy in $CD8^+$ T cells, and not for $CD4^+$ T cells. The enhanced effect on T cell proliferation was transient and resolved after treatment discontinuation. Interestingly, the percentage of Ki-67 decreased on the subsequent time points after the discontinuation of the treatments in $CD8^+$ T cells when mice treated with IL-7 and combined therapy compared to the PBS treated mice **Figure 3-12D**.


PBS 200ml/mouse /injection n=4
CD127 3.5mg/mouse/injection n=3
IL-7 1mg/mouse/injection n=3
Combined treatment 4.5mg/mouse/injection n=3
A
The percentage of Ki-67+ CD4+ T cells
% Ki-67+ CD4+ T cells
60
50
40
30
20
10
0
D-14
D7
D14
D21
D28
D35
D42
The percentage of Ki-67+ CD8+ T cells
B
% Ki-67+ CD8+ T cells
60
50
40
30
20
10
0
D-14
D7
D14
D21
D28
D35
D42

C


PBS 200ml/mouse /injection n=4
CD127 5mg/mouse/injection n=4
IL-7 5mg/mouse/injection n=4
Combined treatment 10mg/mouse/injection n=4
% Ki-67+ CD4+ T cells
80
60
40
20
0
***
***
D-3
D7
D14
D22
D29
D36
D43

D

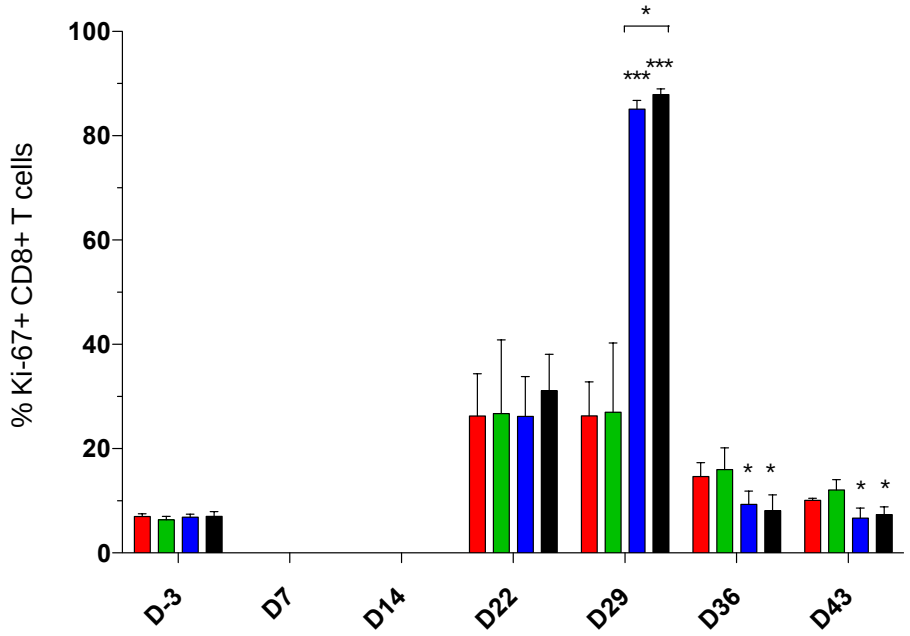

% Ki-67+ CD8+ T cells
100
80
60
40
20
0
*
***
***
* *
* *
D-3
D7
D14
D22
D29
D36
D43

Figure 3-12: The effect of sCD127 treatment on blood T cell proliferation in T cell depleted WT C57BL/6 mice. A/B) The effect of low, two consecutive day single doses of sCD127 and/or IL-7 on T cell proliferation in the blood of depleted C57BL/6 mice. Mice were divided into 4 groups, and treated twice with either 1 μg of IL-7, 3.5 μg of sCD127, 4.5 μg of IL-7 and sCD127 complexed (pre-incubated together for 30 mins at 37°C), or 200 μl PBS alone (vehicle control). The graphs show the frequency of Ki67+ cells amongst $CD4^+$ **(A)** and $CD8^+$ **(B)** T cells. N=3 mice per treatment groups and n=4 mice per PBS group. **C/D)** The effect of daily high dose injections for five consecutive days of sCD127 and/or IL-7 on T cell proliferation in the blood of depleted C57BL/6 mice. Mice were divided into 4 groups, n= 4 mice per group, and treated by i.p. injection for five consecutive days with either: 5 μg of IL-7, 5 μg of sCD127, 10 μg of both treatments complexed (pre-incubated for 30 mins at 37°C), or 200 μl of PBS alone (vehicle control). The graphs show the frequency of Ki67+ cells amongst $CD4^+$ **(C)** and $CD8^+$ **(D)** T cells. Data represent mean ± SD. Statistical analysis was completed using a one-way ANOVA for more than two groups, and a Student's two-tailed unpaired t-test was used for comparisons between two groups. Significant P values are listed as either *P<0.05, ** P<0.05, or ***P<0.001.

4. Chapter 4: Discussion

Soluble cytokine receptors are known to participate in cytokine regulation. In some instances, the soluble cytokine receptors enhance their cognate cytokine signals by preventing degradation or clearance of the cytokine, while others may antagonize or inhibit the effects of the cytokine. While IL-7 signaling plays pivotal roles in the development and homeostasis of T cells, the biological effects of sCD127 in regulating IL-7 signaling in vivo have not been fully determined. Both agonistic and antagonistic roles have been shown for sCD127 toward IL-7 bioactivity. Some studies have reported that sCD127 can inhibit IL-7 signaling (151, 152), while conflicting data have also been provided suggesting enhanced roles of the soluble receptor on IL-7 function (150, 153). Since sCD127 is generally found to circulate at a molar excess relative to IL-7, it suggests that sCD127 in fact can add an extra layer of regulation in IL-7 signaling (85). The essential role of IL-7 in T cell development and homeostasis by maintaining the number of T cells in the periphery at a constant level via enhancing the survival and proliferation of T cell has led to examine IL-7 in clinical trials to treat conditions manifested by lymphopenia as in the case of chemotherapy and AIDS (161–163). Thus, investigating the role of sCD127 in regulating IL-7 functions will not only help to have a better understanding for the biology of sCD127, but also will add additional therapeutic benefits to the use of IL-7 in future clinical applications. The work presented here evaluates the effect of IL-7 on T cell proliferation in healthy mice and the effect of IL-7 on T cell reconstitution in a T cell depletion model. We also assessed whether sCD127 has positive or negative impacts on IL-7 activities. The results reported here reconcile published data from our laboratory demonstrating that combining sCD127 with IL-7 treatments can have a synergistic effect on IL-7 functions, but only under optimal conditions (153). In the event that IL-7 concentrations

are high, the maximal effect of IL-7 would negate sCD127 enhancing IL-7 function any further.

4.1 Effect of Recombinant Murine IL-7 and sCD127 treatment on T cell homeostasis in healthy mice

It is well documented that IL-7 plays a non-redundant role on T cell homeostasis and does so by providing T cells with the survival and proliferative signals needed to maintain T cells at a constant level in the periphery (130). Under homeostatic equilibrium states, the generation and loss of T cells is tightly controlled by various homeostatic mechanisms. These require, in addition to IL-7 signaling, other essential factors including the interaction of TCR on T cells with the self-peptides on APCs and other essential cytokine signals e.g IL-2, 4, and 15 (164, 165). Here we attempt to induce the proliferation of T cells in healthy mice, despite the non-requirement for the process in the steady state environment, by administering exogenous IL-7. Determining the optimal dose of IL-7 required to activate T cell expansion was critical for the experiments aimed at assessing the impact of sCD127 on IL-7 effects. Since IL-7 has been reported to circulate in healthy human plasma at concentrations ranging from 8–10 pg/ml (82), we initiated our first approach of treatment using a single dose of supraphysiological murine IL-7 as indicated above (**Figure 2-1 A)**. This dosing approach did not activate the proliferation of T cells and thus we were not able to reveal any significant change in IL-7 function due to the addition of sCD127. Hence, we speculated that more frequent and higher doses of the treatment would be required to reveal an appreciable effect of IL-7 on T cell proliferation. In the report published by Andersson et al 2011, they reported that administering a 5 μg/dose of either IL-7 alone or in a combination with an equal dose of sCD127 successfully increased the frequency of T cells and the effect was more pronounced

when mice were treated frequently with IL-7 (159). Therefore, we adopted this therapeutic approach in our second attempt for the treatment plan as mentioned previously in **Figure 2-1 B.** One of the first experiments we did to ensure the efficacy of the treatment was to look for a primary signal of successful IL-7 signal transduction. It is known that once IL-7 binds to its target cells, it down-regulates its own receptor on the surface of T cells. It does so as part of intrinsic feedback controls that regulate IL-7 signaling (111, 112). Here we found that administering IL-7 to healthy mice transiently down-regulates the surface expression of CD127 as shown in **Figure 3-2**, and when IL-7 was administered in combination with sCD127 this effect was maintained. Even though no further downregulation was induced when IL-7 was complexed with the soluble receptor, we did not report a negative impact on the IL-7-mediated down-regulation of the membrane-bound CD127. This result contradicts previous reports where sCD127 was found to inhibit IL-7 signal transduction by inhibiting the phosphorylation of STAT5 and AKT which are two of the main downstream signals of IL-7 stimulation (151, 152).

Next, although IL-7 has been shown to induce the homeostatic proliferation of human, primate, and murine T cells, little is known about the contribution of sCD127 to the IL-7 effects (166–169). In this study, we aimed to further elucidate the efficacy of administering murine IL-7 alone or in combination with the sCD127 to induce the proliferation of $CD4^+$ T cells and $CD8^+$ T cells in both mice blood and spleen based on the quantification of Ki-67 levels. In agreement with literature that shows IL-7 promotes T cell proliferation in both depleted and replete hosts, we found that healthy mice treated with IL-7 for 5 consecutive days indeed showed enhanced proliferation of both $CD4^+$ and $CD8^+$ T cells. Further, when IL-7 was administered in combination with sCD127, more pronounced effects were observed **Figure 3-3 C-D and Figure 3-5 A-B**. The results presented here show that sCD127 augments the IL-

7 effect on T cell proliferation in vivo, which is consistent with a previous in vitro study published from our laboratory (153). One of the limitations of this experiment is that it is unknown how sCD127 potentiates IL-7 activity. sCD127 may extend the half-life of IL-7 in vivo, or sCD127 may trans-present the IL-7 to its target cells, which are two of the proposed mechanisms by which soluble receptors can regulate the activity of their cytokine ligands (146–148). As Lundstrom et al proposed, sCD127 potentiates IL-7 function in vivo by the extending its half-life, and further research is needed to determine whether IL-7 trans-presentation is involved or not (150).

In addition to the positive influence on T cell proliferation, we also observed that both blood and spleen T cell numbers increased following IL-7 treatment alone, and further enhancement was achieved by complexing IL-7 with sCD127. It was noteworthy to report that greater responses were observed in $CD8^+$ T cells in that a more pronounced proliferation and significant increase in cell number was observed relative to the effect seen on $CD4^+$ T cells. These findings were consistent with previous reports published by Geiselhart et al and Sportes et al which both showed a similar preferential response of $CD8^+$ T cells toward IL-7 with regards to T cell proliferation compared to $CD4^+$ T cells (167, 168). This suggests that there might be other underlying factors regulating the homeostatic proliferation of $CD4^+$ T cells that accounts for the lesser degree of proliferation in response to IL-7 treatment. To further explain this hypothesis, Guimond et al revealed that elevation of IL-7 level – such as in conditions of lymphopenia or after injection of exogenous IL-7 – down-regulates MHC II expression on APCs, thus indirectly interfering in the TCR-mediated homeostatic proliferation of $CD4^+$ T cells (170). This factor was not tested in our model, and therefore future work would be needed to determine the optimal condition required to enhance $CD4^+$ T cell proliferation, especially in cases where IL-7 levels are found to be elevated.

In regard to the observed increase in T cell number, there are several mechanisms which may contribute to this finding. It may be a thymic-dependent mechanism, where new T cells are exported from the thymus to the periphery. Other mechanisms that could affect T cell numbers independent of the thymus include: a redistribution of T cells from their original homing sites to other locations; proliferation of peripheral T cells; or inhibition of T cell apoptosis. It is unlikely that the increase mediated by IL-7 observed resulted from export from the thymus, as it has been predicted that 3 to 4 weeks is required for the thymus to replenish the periphery with a new wave of mature T cells (171, 172), and we only monitored the mice for a period of one week. Of note, IL-7 has been shown to increase T cell number to similar levels in both normal and thymectomized mice (169). The increased T cell number observed in our experiment is more likely to be attributed to the expansion of naïve T cells compartments in the periphery or through the redistribution of T cells between the different homing sites in the SLOs. As both blood and spleen T cell numbers are elevated after treatments, it is unlikely that T cell redistribution primarily accounts for the relative increase. The possibility that the anti-apoptotic effect of IL-7 serves to maintain the survival of the proliferating cells via the inhibition of apoptosis is supported by a previous report that found that the IL-7-mediated increase in T cell numbers began to dissipate a week following treatment discontinuation (173). Hence, we concluded that both induction of proliferation and inhibition of cell death likely contribute to the accumulation of T cells observed in these experiments.

The experiments on healthy mice aimed to: 1) determine the impact of administering IL-7 and sCD127 on T cell proliferation; and 2) assess the role(s) of IL-7 and sCD127 in activating T cell differentiation. It is noteworthy that our mice were kept in a pathogen-free environment throughout the entire period of our work. Hence, any phenotypic changes reported among $CD4^+$ T cells and $CD8^+$ T cells can be contributed entirely to the treatments.

It has been reported that T_N can be differentiated into T_M in response to the homeostatic proliferation via interaction with self-antigens and IL-7 signals (69). These differentiated memory cells are referred to memory-like phenotype T cells, and have been reported to possess similar functions and express markers resembling that of conventional memory T cells, even though they are not generated by contact with a foreign antigen (70, 71). In our study, the distribution of T cell subsets was distinguished by the expression of CD62L and CD44. Expression levels of these markers have been used by others to characterize T cell phenotypes (66, 174). Here we have demonstrated that in healthy mice IL-7 treatment induces $CD62L^{high}$ $CD44^{low}$ $CD4^+$ T_N cells in the spleen to phenotypically shift to become $CD62L^{low}$ $CD44^{high}$ $CD4^+$ T_E cells **Figure 3-6**. However, in the case of $CD8^+$ T cells, a memory cell-like phenotype characterized by $CD62L^{high}$ $CD44^{high}$ resulted from differentiating $CD62L^{high}$ $CD44^{low}$ $CD8^+$ T_N following IL-7 treatment **Figure 3-7**. Interestingly, however, the combination of sCD127 and IL-7 significantly enhanced the differentiation of T cells in IL-7 treated mice. The result reported here is in contrast to previous in vivo findings demonstrating that IL-7 treatment does not alter phenotypic markers of naïve T cells, nor does it induces T_N to become T_M (174, 175). The effect of IL-7 treatment on T cells appears to be reversible after treatment cessation (176), so whether the differentiation of T cells reported in our model will regain the naïve phenotype as evident before treatment initiation will require further investigation.

4.2 Effects of Recombinant Murine IL-7 and sCD127 treatment on T cell Reconstitution in Depleted Mice

After demonstrating that sCD127 augmented the effect of IL-7-mediated T cell proliferation in healthy mice, we aimed to characterize the results in an experimental model. It is known that T cell proliferation is induced following lymphopenia. Lymphopenia can occur following pathogenic conditions, such as viral infection or septic shock, or from therapeutic

interventions by administering chemotherapy or radiotherapy (177–179). In murine studies, lymphopenia can be induced by various approaches including, but not limited to, genetic manipulation of mice by removing genes essential for T cell development and survival such as recombination activating gene (RAG) and IL-7R genes (70, 180). Our primary goal was to develop a mouse model where T cells would first be depleted, triggering compensatory T cell proliferation that would subsequently lead to a reconstitution of the depleted cells. In this way, T cells would be subjected to a depletion insult that would be followed by a healing period, a model we believe better mimics numerous clinical conditions and preferable to using genetically-modified knockout mice. It has been well documented that IL-7 signaling plays indispensable roles in the homeostatic maintenance of T cells following lymphopenia (180), therefore we were particularly interested in delineating the effects of sCD127 administration on IL-7-mediated T cell reconstitution.

We first validated a mouse model of T cell depletion using wild type C57BL/6 mice treated with specific monoclonal antibodies targeting $CD4^+$ and $CD8^+$ T cells. Different doses of antibodies results in varying severity of T cell depletion. Notably, dosing of as low as 30 μg was associated with significant but partial depletion of the target T cells, whereas treatment doses ranging between 100 and 500 μg were sufficient to deplete most of the T cells in peripheral blood and secondary lymphoid organs (181–183). Therefore, we decided to treat the mice with an equal dose of anti-CD4/anti-CD8 depletion antibodies – 150 μg of each, and confirmed that both T cell subsets were rapidly and completely depleted from mice blood one day following antibody administration (**Figure 3-8**). Interestingly the effect observed in our study was specific to the targeted T cell populations and had minimal off-target effects on other lymphocyte populations examined, specifically B and NK cells (data not shown). These

observations are consistent with the fact that using anti-CD4 and anti-CD8 in combination is more specific with more potent depletion efficacy compared to anti-CD3 antibodies (181).

In our depletion model, we were able to trigger homeostatic proliferation in depleted mice, and delineate the pattern of T cell reconstitution among the different T cell subsets presented herein. Although both $CD4^+$ and $CD8^+$ T cells exhibited a rapid and similar response to the depletion, there was a substantial difference in the kinetics of $CD4^+$ and $CD8^+$ T cell reconstitution **Figure 3-8**. The former was able to recover completely in a period of 6 weeks following T cell depletion **Figure 3-8A-C**, whereas a delayed and incomplete recovery of $CD8^+$ T cells was observed over the same time period **Figure 3-8D-F**. Notably, it took approximately 3 months for $CD8^+$ T cells to return to their basal level (pre-depletion) using this mouse model (data not shown). We therefore decided to stop monitoring the kinetics of T cell reconstitution by 6 weeks as our results for the Ki-67 expression indicated that no active division was observed for the subsequent time points **Figure 3-10**. This finding differs from a previously published report by Engram et al., that showed $CD8^+$ T cells reconstitute more rapidly after antibody-mediated depletion in non-human primate compared with that of $CD4^+$ T cells (184). A similar trend of efficient and faster reconstitution among $CD8^+$ T cell population has also been shown in human subjects following chemotherapy or undergoing hematopoietic stem cell transplantation (185, 186).

One possible explanation accounting for the discrepancy in the reconstitution rate observed might be a result of the use of equal concentrations of the depletion antibodies in our model. The fact that $CD4^+$ T cells make up a higher proportion of T cells than $CD8^+$ T cells might require adjusting the dose of antibody regimens to account for the difference in the composition of the targeted cells. As such, one can speculate that if $CD8^+$ T cells were completely depleted from the periphery, $CD8^+$ T cell reconstitution would require de novo

thymic T cell development, thus delaying the T cell reconstitution. On the other hand, if few residual $CD4^+$ T cells resist antibody-mediated depletion, the $CD4^+$ T cells could be replenished by undergoing homeostatic proliferation, which would be faster than de novo thymic T cell development. Another factor that may explain the different response to the depletion antibodies is the stability of the antibody used to deplete the T cells. The slower rate of $CD8^+$ T cell reconstitution might be caused by the use of a more stable antibody against $CD8^+$ compared to the antibody used against $CD4^+$ T cells. This could have been investigated by monitoring the concentration of the depletion antibodies in mouse serum following the administration, however, this has not been directly tested. Lastly, the source of replenishing T cells after depletion might also play an important factor. Several lines of evidence suggest that thymic-dependent and extrathymic-mediated homeostatic expansion lymphopoiesis are responsible for the different patterns of T cell regeneration which differs between $CD4^+$ and $CD8^+$ T cells (187–189). One limitation of this study is that we could not delineate the source of the T cells recovered following the depletion, and further experiments would be required to do so.

Lastly, this study aimed to determine the effect of administering murine IL-7 alone or in combination with the sCD127 to enhance the reconstitution of $CD4^+$ T cells and $CD8^+$ T cells in mice following T cell depletion. Similar to the dual therapeutic approaches used in healthy mice, mice undergoing T cell depletion received one of two treatment plans with a slightly different dosing frequency from healthy mice **(See Figure 2-3 A-B).** In the first approach, T cell depleted mice were treated with a low concentration (of IL-7, sCD127 or both) and only two treatment injections. Consistent with the negative results obtained from healthy mice, this low dose approach in depleted mice also did not activate the proliferation of T cells and had no impact on overall T cell reconstitution (**See Figure 3-11A-C and Figure**

3-12A-B). However, repeating the experiments with more frequent and higher doses of the treatment revealed significant effects suggesting enhanced T cell recovery (**See Figure 3-11E-G and Figure 3-12C-D**). Again, as seen in the non-depleted model, $CD8^+$ T cells exert preferential responses to our treatments relative to the effect seen on $CD4^+$ T cells. It is also noteworthy that we were not able to demonstrate a statistically significant difference between IL-7 treatment alone and the combination treatments, as IL-7 treatment likely resulted in maximal T cell proliferation. Therefore, titrating IL-7 concentration is required first before assessing if there can be an appreciable impact of sCD127 on T cell proliferation when combined with a sub-maximal dose of IL-7. Another interesting observation was that both treatments had a transient effect on T cell proliferation and reconstitution. The effect of IL-7 was completely reversible after treatment discontinuation in that the total number of Ki-67-positive $CD4^+$ and $CD8^+$ T cells reverted to the level of control group (PBS treated mice) (**See Figure 3-12C-D**). This finding is in line with previous reports showing the same observations and all together suggested continuous treatment with IL-7 would required to achieve more rapid recovery of the depleted T cells (174, 176).

It is evident that elevated IL-7 levels have been associated with several lymphopenic conditions (190, 191). However, our attempts to determine how IL-7 concentrations would change following T cell depletion were not successful (**See Figure 3-9**). This was possibly due to the limitation of our study given the small the plasma volumes collected for this purpose. As per University of Ottawa animal handling guidelines, we were limited to obtaining approximately 100 µl of blood per mouse weekly. This volume was subsequently split in half: half for blood cell analysis by flow cytometry, and half quantification of IL-7 in plasma. The total plasma volume from each mouse acquired ranged between 20-30 µl, making it difficult to detect a cytokine like IL-7 that is <10 pg/ml in mouse blood. Therefore, optimizing an

antibody-mediated T cell depletion model in mice with an enhanced IL-7 quantification method would be required in future experiments to have a better understanding of the biology of this model.

5. Future directions for targeting sCD127 as a modulator of IL-7 in disease models

Clinical development of IL-7 is currently underway as its therapeutic potential has been examined in various animal models and human clinical trials (127). The most obvious clinical application of IL-7 is to enhance immune reconstitution during lymphopenic conditions such as those in cancer patients receiving chemotherapy or following infectious diseases such as immunodeficiency-induced by HIV. As already discussed, sCD127 has been shown to enhance the effect of IL-7-mediated increases in both T cell numbers and proliferation (153). Therefore, combination therapy with IL-7 holds promise as an alternative treatment that could provide greater benefit than IL-7 alone for patients with profound T cell depletion. This may be especially important in the setting of regenerating T cells following bone marrow transplantation (BMT) (192), as diminished immune reconstitution characterized by abnormal proportions of $CD4^+$ and $CD8^+$ T cells. Impaired T cell proliferation in response to mitogens also remain obstacles for successful stem cell transplantation (193, 194). Since IL-7 in our experiments induces T cell proliferation in both healthy and T cell depleted mice, and subsequent combination with sCD127 further enhanced IL-7 functions, a combination therapy proposed herein may potentially be used to enhance lymphocyte reconstitution following BMT.

Another possible application of combination therapy is to improve the efficacy of immunotherapy following adaptive T cell transfer to treat malignancies or chronic infections. It is known that sustained immunological responses manifested by potent $CD8^+$ T cells can mediate cytotoxicity and development of long-lived memory T cells that are of particular interest for successful treatment. The results presented here showed that combination therapy has the potential to stimulate T cell differentiation resulting in the development of more memory T cells compared to that seen when mice were treated with IL-7 alone. This could

therefore make combination therapy a promising candidate immune adjuvant therapy for subsequent studies aimed to enhance memory T cell formation. Moreover, a previous report from our laboratory showed that enhanced $CD8^+$ T cell responses, characterized by the release of higher amounts of IFN-γ, were more pronounced when human and mice $CD8^+$ T cells were treated with the combination therapy compared to IL-7 alone (153). These data are encouraging and indicate combining IL-7 with sCD127 may be used in subsequent models aimed to enhance the cytotoxic effect of $CD8^+$ T cells.

6. Concluding Remarks

This study has demonstrated that in healthy mice treatment with combination of sCD127 and IL-7 enhances IL-7-mediated $CD4^+$ and $CD8^+$ T cells proliferation, increased their cell numbers, and induced the differentiation of naïve T cell compartments. Of note, $CD8^+$ T cells exert preferential responses to the treatments in that a more pronounced proliferation and significant increase in cell number was observed relative to the effect seen on $CD4^+$ T cells in both healthy and depleted mice. For T cell depletion experiments, this is the first time to the best of our knowledge that the kinetics of $CD4^+$ and $CD8^+$ T cell reconstitution after antibody-mediated T cell depletion in mice has been performed. The main findings for the depletion model are the following: (1) both anti-CD4/anti-CD8 depletion antibodies induced a rapid, profound, and complete depletion of $CD4^+$ and $CD8^+$ T cells in mice blood one day following the antibody administration; (2) $CD4^+$ and $CD8^+$ lymphocyte depletion was followed by a rapid proliferation as quantified by Ki-67 expression which was at its highest when cells were mostly depleted, and returned to its basal expression 6 weeks following depletion; (3) the reconstitution of $CD4^+$ T cells was significantly higher and faster than that of $CD8^+$ T cells, despite the use of equal antibody concentration; and (4) Following T cell depletion, we were not able to report any increase in the mouse plasma levels of IL-7 that might correlate with the activation of T cell proliferation during the reconstitution phase. We also concluded that treatment with IL-7 with or without sCD127 had a transient and significant effect on T cell proliferation and reconstitution following depletion, and this influence resolved after treatment discontinuation. Overall, work presented here will not only help to have a better understanding for the biology of sCD127 in regulating IL-7 functions, but also will provide insight into potential therapeutic benefits to the use of IL-7 in future clinical applications.

References

1. Rodríguez RM, López-Vázquez A, López-Larrea C. 2012. Immune systems evolution. Adv Exp Med Biol.
2. Famili F, Wiekmeijer A-S, Staal FJ. 2017. The development of T cells from stem cells in mice and humans. Futur Sci OA.
3. Moser M, Leo O. 2010. Key concepts in immunology. Vaccine 28:2–13.
4. Maldonado-Contreras AL, McCormick BA. 2011. Intestinal epithelial cells and their role in innate mucosal immunity. Cell Tissue Res.
5. Gallo RL, Murakami M, Ohtake T, Zaiou M. 2002. Biology and clinical relevance of naturally occurring antimicrobial peptides. J Allergy Clin Immunol.
6. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. 2012. PAMPs and DAMPs: Signal 0s that spur autophagy and immunity. Immunol Rev.
7. Jang JH, Shin HW, Lee JM, Lee HW, Kim EC, Park SH. 2015. An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. Mediators Inflamm.
8. Janeway CA, Medzhitov R. 2002. Innate Immune Recognition. Annu Rev Immunol.
9. Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. 1999. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by Toll-like receptor 2. J Biol Chem.
10. Kawai T, Akira S. 2010. The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. Nat Immunol.
11. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. 2001. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature.
12. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. 2001. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature.
13. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. 2001. A Toll-like receptor recognizes bacterial DNA (vol 408, pg 740, 2000). Nature.
14. Aderem A, Ulevitch RJ. 2000. Toll-like receptors in the induction of the innate immune response. Nature.
15. Sarma JV, Ward P a. 2012. The Compliment System. NIH Public Access author Manuscr.
16. Sonnen AFP, Henneke P. 2014. Structural biology of the membrane attack complex. Subcell Biochem 80:83–116.
17. Kinchen JM, Ravichandran KS. 2008. Phagosome maturation: Going through the acid test. Nat Rev Mol Cell Biol.
18. Jiang H, Chess L. 2009. Chapter 2 How the Immune System Achieves Self–Nonself Discrimination During Adaptive Immunity. Adv Immunol 102:95–133.
19. Market E, Papavasiliou FN. 2003. V(D)J Recombination and the Evolution of the Adaptive Immune System. PLoS Biol.
20. Ollila J, Mauno V. 2005. B cells. Int J Biochem Cell Biol 33:518–523.
21. Vyas JM, Van Der Veen AG, Ploegh HL. 2008. The known unknowns of antigen processing and presentation. Nat Rev Immunol.
22. Neefjes J, Jongsma MLM, Paul P, Bakke O. 2011. Towards a systems understanding of MHC class i and MHC class II antigen presentation. Nat Rev Immunol.

23. Tanel A, Fonseca SG, Yassine-Diab B, Bordi R, Zeidan J, Shi Y, Benne C, Sékaly R-P. 2009. Cellular and molecular mechanisms of memory T-cell survival. Expert Rev Vaccines 8:299–312.
24. Brummendorf TH, Dragowska W, Zijlmans JMJM JMJM, Thornbury G, Lansdorp PM. 1998. Asymmetric cell divisions sustain long-term hematopoiesis from single-sorted human fetal liver cells. J Exp Med.
25. Petrie HT, Zúñiga-Pflücker JC. 2007. Zoned Out: Functional Mapping of Stromal Signaling Microenvironments in the Thymus. Annu Rev Immunol.
26. Godfrey DI, Kennedy J, Suda T, Zlotnik A. 1993. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J Immunol.
27. Capone M, Hockett RD, Zlotnik A. 1998. Kinetics of T cell receptor , , and rearrangements during adult thymic development: T cell receptor rearrangements are present in CD44+CD25+ Pro-T thymocytes. Proc Natl Acad Sci.
28. Groettrup M, Ungewiss K, Azogui O, Palacios R, Owen MJ, Hayday AC, Boehmer H von. 1993. A novel disulfide-linked heterodimer on pre-T cells consists of the T cell receptor β chain and a 33 kd glycoprotein. Cell.
29. Fahl SP, Coffey F, Wiest DL. 2014. Origins of γδ T cell effector subsets: a riddle wrapped in an enigma. J Immunol 193:4289–4294.
30. Starr TK, Jameson SC, Hogquist KA. 2003. Positive and negative T cell selection. Annu Rev Immunol.
31. Förster R, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M. 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell.
32. Mester G, Hoffmann V, Stevanović S. 2011. Insights into MHC class I antigen processing gained from large-scale analysis of class I ligands. Cell Mol Life Sci.
33. Bryant P, Ploegh H. 2004. Class II MHC peptide loading by the professionals. Curr Opin Immunol.
34. Cresswell P. 1996. Invariant chain structure and MHC class II function. Cell.
35. Sherman MA, Weber DA, Jensen PE. 1995. DM enhances peptide binding to class II MHC by release of invariant chain-derived peptide. Immunity.
36. Broere F, Apasov SG, Sitkovsky M V., van Eden W. 2011. A2 T cell subsets and T cell-mediated immunity, p. 15–27. *In* Principles of Immunopharmacology. Birkhäuser Basel.
37. Malek TR. 2008. The Biology of Interleukin-2. Annu Rev Immunol 26:453–479.
38. Zhu J, Yamane H, Paul WE. 2010. Differentiation of Effector CD4 T Cell Populations. Annu Rev Immunol 28:445–489.
39. Saravia J, Chapman NM, Chi H. 2019. Helper T cell differentiation. Cell Mol Immunol 16:634–643.
40. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. 1993. Development of T H 1 CD4 + T cells through IL-12 produced by Listeria-induced macrophages. Science (80-) 260:547–549.
41. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. 2000. A Novel Transcription Factor, T-bet, Directs Th1 Lineage Commitment. Cell 100:655–669.
42. Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DAA, Doherty PC, Grosveld GC, Ihle JN. 1996. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature 382:171–174.

43. Finkelman FD, Shea-Donohue T, Morris SC, Gildea L, Strait R, Madden KB, Schopf L, Urban Jr JF. 2004. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. Immunol Rev 201:139–155.
44. Zheng W, Flavell RA. 1997. The Transcription Factor GATA-3 Is Necessary and Sufficient for Th2 Cytokine Gene Expression in CD4 T Cells. Cell 89:587–596.
45. Stockinger B, Veldhoen M. 2007. Differentiation and function of Th17 T cells. Curr Opin Immunol 19:281–286.
46. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. 2006. Transforming growth factor-β induces development of the TH17 lineage. Nature 441:231–234.
47. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, McClanahan TK, O'Shea JJ, Cua DJ. 2009. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17–producing effector T helper cells in vivo. Nat Immunol 10:314.
48. Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, Monticelli S, Lanzavecchia A, Sallusto F. 2012. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. Nature 484:514.
49. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. 2007. IL-6 programs TH-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 8:967.
50. Huang W, Na L, Fidel PL, Schwarzenberger P. 2004. Requirement of Interleukin-17A for Systemic Anti-Candida albicans Host Defense in Mice. J Infect Dis 190:624–631.
51. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, Schwarzenberger P, Shellito JE, Kolls JK. 2001. Interleukin-17 and Lung Host Defense againstKlebsiella pneumoniae Infection. Am J Respir Cell Mol Biol 25:335–340.
52. Uyttenhove C, Van Snick J. 2006. Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis. Eur J Immunol 36:2868–2874.
53. Röhn TA, Jennings GT, Hernandez M, Grest P, Beck M, Zou Y, Kopf M, Bachmann MF. 2006. Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis. Eur J Immunol 36:2857–2867.
54. Littman DR, Rudensky AY. 2010. Th17 and Regulatory T Cells in Mediating and Restraining Inflammation. Cell 140:845–858.
55. Fontenot JD, Gavin MA, Rudensky AY. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4:330–336.
56. Walch M, Latinovic-Golic S, Velic A, Sundstrom H, Dumrese C, Wagner CA, Groscurth P, Ziegler U. 2007. Perforin enhances the granulysin-induced lysis of Listeria innocua in human dendritic cells. BMC Immunol 8:14.
57. Endsley JJ, Torres AG, Gonzales CM, Kosykh VG, Motin VL, Peterson JW, Estes DM, Klimpel GR. 2009. Comparative antimicrobial activity of granulysin against bacterial biothreat agents. Open Microbiol J 3:92–96.
58. Kelly JM, Waterhouse NJ, Cretney E, Browne KA, Ellis S, Trapani JA, Smyth MJ. 2004. Granzyme M mediates a novel form of perforin-dependent cell death. J Biol Chem 279:22236–42.
59. Yasukawa M, Ohminami H, Arai J, Kasahara Y, Ishida Y, Fujita S. 2000. Granule exocytosis, and not the Fas/Fas ligand system, is the main pathway of cytotoxicity mediated by alloantigen-specific CD4⁺ as well as CD8⁺ cytotoxic T lymphocytes in humans. Blood 95:2352 LP – 2355.

60. Badovinac VP, Porter BB, Harty JT. 2002. Programmed contraction of CD8+ T cells after infection. Nat Immunol 3:619–626.
61. Schluns KS, Williams K, Ma A, Zheng XX, Lefrançois L. 2002. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. J Immunol 168:4827–4831.
62. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. 2000. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. Nat Immunol 1:426.
63. Kaech SM, Hemby S, Kersh E, Ahmed R. 2002. Molecular and Functional Profiling of Memory CD8 T Cell Differentiation. Cell 111:837–851.
64. Opferman JT, Ober BT, Ashton-Rickardt PG. 1999. Linear Differentiation of Cytotoxic Effectors into Memory T Lymphocytes. Science (80-) 283:1745–1748.
65. Manjunath N, Shankar P, Wan J, Weninger W, Crowley MA, Hieshima K, Springer TA, Fan X, Shen H, Lieberman J, von Andrian UH. 2001. Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. J Clin Invest 108:871–878.
66. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401:708.
67. Ernst B, Lee D-S, Chang JM, Sprent J, Surh CD. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. Immunity 11:173–181.
68. Goldrath AW, Bevan MJ. 1999. Low-affinity ligands for the TCR drive proliferation of mature CD8+ T cells in lymphopenic hosts. Immunity 11:183–190.
69. Kieper WC, Jameson SC. 1999. Homeostatic expansion and phenotypic conversion of naive T cells in response to self peptide/MHC ligands. Proc Natl Acad Sci 96:13306–13311.
70. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. 2000. Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. J Exp Med 192:549–556.
71. Goldrath AW, Bogatzki LY, Bevan MJ. 2000. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. J Exp Med 192:557–564.
72. Hamilton SE, Wolkers MC, Schoenberger SP, Jameson SC. 2006. The generation of protective memory-like CD8+ T cells during homeostatic proliferation requires CD4+ T cells. Nat Immunol 7:475.
73. Ferreira VL, Borba HHL, Bonetti ADF, Leonart L, Pontarolo R. 2018. Cytokines and interferons: types and functionsAutoantibodies and Cytokines.IntechOpen. IntechOpen.
74. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, Klunker S, Meyer N, O'Mahony L, Palomares O. 2011. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. J Allergy Clin Immunol 127:701–721.
75. Catalan-Dibene J, Vazquez MI, Nuccio S-P, Karimzadeh A, Kastenschmidt JM, Villalta SA, Ushach I, Pone EJ, Casali P, Raffatellu M. 2017. Identification of IL-40, a Novel B Cell–Associated Cytokine. J Immunol 199:3326–3335.
76. Wang X, Wei Y, Xiao H, Liu X, Zhang Y, Han G, Chen G, Hou C, Ma N, Shen B. 2016. A novel IL-23p19/Ebi3 (IL-39) cytokine mediates inflammation in lupus-like mice. Eur J Immunol 46:1343–1350.
77. Sim GC, Radvanyi L. 2014. The IL-2 cytokine family in cancer immunotherapy. Cytokine Growth Factor Rev 25:377–390.

78. Jiang Q, Wen QL, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, Durum SK. 2005. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev 16:513–533.
79. Namen AE, Lupton S, Hjerrild K, Wignall J, Mochizuki DY, Schmierer A, Mosley B, March CJ, Urdal D, Gillis S, Cosman D, Goodwin RG. 1988. Stimulation of B-cell progenitors by cloned murine interleukin-7. Nature.
80. von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. 2004. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med.
81. Mazzucchelli R, Durum SK. 2007. Interleukin-7 receptor expression: Intelligent design. Nat Rev Immunol 7:144–154.
82. Fry TJ, Connick E, Falloon J, Lederman MM, Liewehr DJ, Spritzler J, Steinberg SM, Wood L V., Yarchoan R, Zuckerman J, Landay A, Mackall CL. 2001. A potential role for interleukin-7 in T-cell homeostasis. Blood 97:2983–2990.
83. Lynch M, Baker E, Park LS, Sutherland GR, Goodwin RG. 1992. The interleukin-7 receptor gene is at 5pl3. Hum Genet 89:566–568.
84. Tal N, Shochat C, Geron I, Bercovich D, Izraeli S. 2014. Interleukin 7 and thymic stromal lymphopoietin: from immunity to leukemia. Cell Mol life Sci 71:365–378.
85. Janot-Sardet C, Assouline B, Cheynier R, Morre M, Beq S. 2010. A validated assay to measure soluble IL-7 receptor shows minimal impact of IL-7 treatment. J Immunol Methods 353:115–123.
86. Slyvka AV, Okunev OV. 2014. Molecular mechanisms of versatile biological activity of interleukin-7. Biopolym cell 349–357.
87. Tani-ichi S, Shimba A, Wagatsuma K, Miyachi H, Kitano S, Imai K, Hara T, Ikuta K. 2013. Interleukin-7 receptor controls development and maturation of late stages of thymocyte subpopulations. Proc Natl Acad Sci.
88. Goodwin RG, Friend D, Ziegler SF, Jerzy R, Falk BA, Gimpel S, Cosman D, Dower SK, March CJ, Namen AE, Park LS. 1990. Cloning of the human and murine interleukin-7 receptors: Demonstration of a soluble form and homology to a new receptor superfamily. Cell 60:941–951.
89. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, Ziegler SF, Leonard WJ, Lodish HF. 2000. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. Nat Immunol 1:59.
90. Higuchi M, Asao H, Tanaka N, Oda K, Takeshita T, Nakamura M, Van Snick J, Sugamura K. 1996. Dispensability of Jak1 tyrosine kinase for interleukin-2-induced cell growth signaling in a human T cell line. Eur J Immunol 26:1322–1327.
91. Suzuki K, Nakajima H, Saito Y, Saito T, Leonard WJ, Iwamoto I. 2000. Janus kinase 3 (Jak3) is essential for common cytokine receptor γ chain (γc)-dependent signaling: comparative analysis of γc, Jak3, and γc and Jak3 double-deficient mice. Int Immunol 12:123–132.
92. Smiers F, Pouwels K, Hoefsloot LH, Löwenberg B, Touw IP. 1996. Interleukin-7 signaling in human B cell precursor acute lymphoblastic leukemia cells and murine BAF3 cells involves activation of STAT1 and STAT5 mediated via the interleukin-7 receptor alpha chain. Leukemia 10:1317–1325.
93. Chou W-C, Levy DE, Lee C-K. 2006. STAT3 positively regulates an early step in B-cell development. Blood 108:3005–3011.
94. Heltemes-Harris LM, Willette MJL, Vang KB, Farrar MA. 2011. The role of STAT5 in the development, function, and transformation of B and T lymphocytes. Ann N Y Acad Sci 1217:18–31.

95. Yao Z, Cui Y, Watford WT, Bream JH, Yamaoka K, Hissong BD, Li D, Durum SK, Jiang Q, Bhandoola A. 2006. Stat5a/b are essential for normal lymphoid development and differentiation. Proc Natl Acad Sci 103:1000–1005.
96. Dadi H, Ke S, Roifman CM. 1994. Activation of phosphatidylinositol-3 kinase by ligation of the interleukin-7 receptor is dependent on protein tyrosine kinase activity. Blood 84:1579–1586.
97. Sharfe N, Dadi HK, Roifman CM. 1995. JAK3 protein tyrosine kinase mediates interleukin-7-induced activation of phosphatidylinositol-3' kinase. Blood 86:2077–2085.
98. Downward J. 2004. PI 3-kinase, Akt and cell survival, p. 177–182. *In* Seminars in cell & developmental biology. Elsevier.
99. Kane LP, Weiss A. 2003. The PI-3 kinase/Akt pathway and T cell activation: pleiotropic pathways downstream of PIP3. Immunol Rev 192:7–20.
100. Aiello FB, Keller JR, Klarmann KD, Dranoff G, Mazzucchelli R, Durum SK. 2007. IL-7 Induces Myelopoiesis and Erythropoiesis. J Immunol.
101. Swainson L, Kinet S, Mongellaz C, Sourisseau M, Henriques T, Taylor N. 2007. IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Blood 109:1034–1042.
102. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature.
103. Jicha DL. 1991. Interleukin 7 generates antitumor cytotoxic T lymphocytes against murine sarcomas with efficacy in cellular adoptive immunotherapy. J Exp Med.
104. Sun JC, Lehar SM, Bevan MJ. 2006. Augmented IL-7 signaling during viral infection drives greater expansion of effector T cells but does not enhance memory. J Immunother 177:4458–63.
105. Pillai M, Torok-Storb B, Iwata M. 2004. Expression and function of IL-7 receptors in marrow stromal cells. Leuk Lymphoma.
106. Puel A, Ziegler SF, Buckley RH, Leonard WJ. 1998. Defective IL7R expression in T-B+NK+ severe combined immunodeficiency. Nat Genet.
107. Hoe E, McKay FC, Schibeci SD, Gandhi K, Heard RN, Stewart GJ, Booth DR. 2010. Functionally Significant Differences in Expression of Disease-Associated IL-7 Receptor α Haplotypes in CD4 T Cells and Dendritic Cells. J Immunol 184:2512–2517.
108. Gonzalez-Quintial R, Lawson BR, Scatizzi JC, Craft J, Kono DH, Baccala R, Theofilopoulos AN. 2011. Systemic autoimmunity and lymphoproliferation are associated with excess IL-7 and inhibited by IL-7Rα blockade. PLoS One 6.
109. Digel W, Schmid M, Heil G, Conrad P, Gillis S PF. 1991. Human interleukin-7 induces proliferation of neoplastic cells from chronic lymphocytic leukemia and acute leukemias. Blood 78:753–9.
110. Napolitano LA, Grant RM, Deeks SG, Schmidt D, De Rosa SC, Herzenberg LA, Herndier BG, Andersson J, Mccune JM. 2001. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: Implications for T-cell homeostasis. Nat Med.
111. Palmer MJ, Mahajan VS, Trajman LC, Irvine DJ, Lauffenburger DA, Chen J. 2008. Interleukin-7 receptor signaling network: An integrated systems perspective. Cell Mol Immunol 5:79–89.
112. Alves NL, van Leeuwen EMM, Derks IAM, van Lier RAW. 2008. Differential Regulation of Human IL-7 Receptor Expression by IL-7 and TCR Signaling. J Immunol 180:5201–5210.

113. Grabstein KH, Waldschmidt TJ, Finkelman FD, Hess BW, Alpert AR, Boiani NE, Namen AE, Morrissey PJ. 1993. Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. J Exp Med 178:257–264.
114. Plum J, De Smedt M, Leclercq G, Verhasselt B, Vandekerckhove B. 1996. Interleukin-7 is a critical growth factor in early human T-cell. Blood 88:4239–4245.
115. Puel A, Leonard WJ. 2000. Mutations in the gene for the IL-7 receptor result in T-B+NK+ severe combined immunodeficiency disease. Curr Opin Immunol. Current Biology Ltd.
116. Al-Shami A, Spolski R, Kelly J, Fry T, Schwartzberg PL, Pandey A, Mackall CL, Leonard WJ. 2004. A role for thymic stromal lymphopoietin in CD4+ T cell development. J Exp Med 200:159–168.
117. Fry TJ, Mackall CL. 2002. Interleukin-7: from bench to clinic. Blood 99:3892–3904.
118. Fry TJ, Mackall CL. 2005. The Many Faces of IL-7: From Lymphopoiesis to Peripheral T Cell Maintenance. J Immunol 174:6571–6576.
119. Kim K, Lee C, Sayers TJ, Muegge K, Durum SK. 1998. The trophic action of IL-7 on pro-T cells: inhibition of apoptosis of pro-T1,-T2, and-T3 cells correlates with Bcl-2 and Bax levels and is independent of Fas and p53 pathways. J Immunol 160:5735–5741.
120. Maraskovsky E, O'Reilly LA, Teepe M, Corcoran LM, Peschon JJ, Strasser A. 1997. Bcl-2 can rescue t lymphocyte development in interleukin-7 receptor–deficient mice but not in mutant rag-1−/− mice. Cell 89:1011–1019.
121. Akashi K, Kondo M, von Freeden-Jeffry U, Murray R, Weissman IL. 1997. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor–deficient mice. Cell 89:1033–1041.
122. Khaled AR, Li WQ, Huang J, Fry TJ, Khaled AS, Mackall CL, Muegge K, Young HA, Durum SK. 2002. Bax deficiency partially corrects interleukin-7 receptor α deficiency. Immunity 17:561–573.
123. Pallard C, Stegmann APA, Van Kleffens T, Smart F, Venkitaraman A, Spits H. 1999. Distinct roles of the phosphatidylinositol 3-kinase and STAT5 pathways in IL-7-mediated development of human thymocyte precursors. Immunity 10:525–535.
124. Hagenbeek TJ, Naspetti M, Malergue F, Garçon F, Nunès JA, Cleutjens KBJM, Trapman J, Krimpenfort P, Spits H. 2004. The loss of PTEN allows TCR αβ lineage thymocytes to bypass IL-7 and Pre-TCR–mediated signaling. J Exp Med 200:883–894.
125. Schlissel MS, Durum SD, Muegge K. 2000. The interleukin 7 receptor is required for T cell receptor γ locus accessibility to the V (D) J recombinase. J Exp Med 191:1045–1050.
126. Muegge K, Vila MP, Durum SK. 1993. Interleukin-7: a cofactor for V (D) J rearrangement of the T cell receptor beta gene. Science (80-) 261:93–95.
127. MacKall CL, Fry TJ, Gress RE. 2011. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol 11:330–342.
128. Rathmell JC, Farkash EA, Gao W, Thompson CB. 2001. IL-7 enhances the survival and maintains the size of naive T cells. J Immunol 167:6869–6876.
129. Viret C, Wong FS, Janeway Jr CA. 1999. Designing and maintaining the mature TCR repertoire: the continuum of self-peptide: self-MHC complex recognition. Immunity 10:559–568.
130. Tan JT, Dudl E, LeRoy E, Murray R, Sprent J, Weinberg KI, Surh CD. 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc Natl Acad Sci 98:8732–8737.

131. Vivien L, Benoist C, Mathis D. 2001. T lymphocytes need IL-7 but not IL-4 or IL-6 to survive in vivo. Int Immunol 13:763–768.
132. Li WQ, Jiang Q, Khaled AR, Keller JR, Durum SK. 2004. Interleukin-7 inactivates the pro-apoptotic protein bad promoting T cell survival. J Biol Chem 279:29160–29166.
133. Li WQ, Guszczynski T, Hixon JA, Durum SK. 2010. Interleukin-7 regulates Bim proapoptotic activity in peripheral T-cell survival. Mol Cell Biol 30:590–600.
134. Carrette F, Surh CD. 2012. IL-7 signaling and CD127 receptor regulation in the control of T cell homeostasis. Semin Immunol 24:209–217.
135. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. 2005. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 120:237–248.
136. Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC. 2008. IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 111:2101–2111.
137. Kittipatarin C, Khaled AR. 2007. Interlinking interleukin-7. Cytokine 39:75–83.
138. Li WQ, Jiang Q, Aleem E, Kaldis P, Khaled AR, Durum SK. 2006. IL-7 promotes T cell proliferation through destabilization of p27Kip1. J Exp Med 203:573–582.
139. Beq S, Nugeyre M-T, Fang RHT, Gautier D, Legrand R, Schmitt N, Estaquier J, Barré-Sinoussi F, Hurtrel B, Cheynier R. 2006. IL-7 induces immunological improvement in SIV-infected rhesus macaques under antiviral therapy. J Immunol 176:914–922.
140. Levy Y, Lacabaratz C, Weiss L, Viard J-P, Goujard C, Lelièvre J-D, Boué F, Molina J-M, Rouzioux C, Avettand-Fénoêl V. 2009. Enhanced T cell recovery in HIV-1–infected adults through IL-7 treatment. J Clin Invest 119:997–1007.
141. Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL. 1985. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. J Immunol.
142. Levine SJ. 2004. Generation Mechanisms of Soluble Cytokine Receptor Mechanisms of Soluble Cytokine Receptor Generation. J Immunol Ref 1735343:5343–5348.
143. Fernandez-Botran R, Chilton PM, Ma Y. 1996. Soluble cytokine receptors: Their roles in immunoregulation, disease, and therapy. Adv Immunol. Academic Press Inc.
144. Cabrera R, Ararat M, Cao M, Xu Y, Wasserfall C, Atkinson MA, Liu C, Nelson DR. 2010. Hepatocellular carcinoma immunopathogenesis: clinical evidence for global T cell defects and an immunomodulatory role for soluble CD25 (sCD25). Dig Dis Sci 55:484–495.
145. Arend WP, Malyak M, Smith MF, Whisenand TD, Slack JL, Sims JE, Giri JG, Dower SK. 1994. Binding of IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. J Immunol 153:4766–74.
146. Fernandez-Botran R, Vitetta ES. 1991. Evidence that natural murine soluble interleukin 4 receptors may act as transport proteins. J Exp Med 174:673–681.
147. Stoklasek TA, Schluns KS, Lefrançois L. 2006. Combined IL-15/IL-15Rα immunotherapy maximizes IL-15 activity in vivo. J Immunol 177:6072–6080.
148. Heaney ML, Golde DW. 1998. Soluble receptors in human disease. J Leukoc Biol 64:135–146.
149. Rose T, Lambotte O, Pallier C, Delfraissy J-F, Colle J-H. 2009. Identification and biochemical characterization of human plasma soluble IL-7R: lower concentrations in HIV-1-infected patients. J Immunol 182:7389–7397.

150. Lundstrom W, Highfill S, Walsh STR, Beq S, Morse E, Kockum I, Alfredsson L, Olsson T, Hillert J, Mackall CL. 2013. Soluble IL7R potentiates IL-7 bioactivity and promotes autoimmunity. Proc Natl Acad Sci 110:E1761–E1770.
151. Crawley AM, Faucher S, Angel JB. 2010. Soluble IL-7R (sCD127) Inhibits IL-7 Activity and Is Increased in HIV Infection. J Immunol 184:4679–4687.
152. Monti P, Brigatti C, Krasmann M, Ziegler AG, Bonifacio E. 2013. Concentration and activity of the soluble form of the interleukin-7 receptor a in type 1 diabetes identifies an interplay between hyperglycemia and immune function. Diabetes 62:2500–2508.
153. Côté S, Matte J, Sad S, Angel JB, Crawley AM. 2015. Complexed soluble IL-7 receptor α and IL-7 increase IL-7-mediated proliferation and viability of $CD8^+$ T-cells in vitro. Cell Immunol 293:122–125.
154. Badot V, Luijten RK, van Roon JA, Depresseux G, Aydin S, Van den Eynde BJ, Houssiau FA, Lauwerys BR. 2013. Serum soluble interleukin 7 receptor is strongly associated with lupus nephritis in patients with systemic lupus erythematosus. Ann Rheum Dis 72:453–456.
155. Santiago JL, Alizadeh BZ, Martínez A, Espino L, de la Calle H, Fernández-Arquero M, Figueredo MA, de la Concha EG, Roep BO, Koeleman BPC, Urcelay E. 2008. Study of the association between the CAPSL-IL7R locus and type 1 diabetes. Diabetologia 51:1653–1658.
156. Heron M, Grutters JC, van Moorsel CHM, Ruven HJT, Huizinga TWJ, van der Helm-van Mil AHM, Claessen AME, van den Bosch JMM. 2009. Variation in IL7R predisposes to sarcoid inflammation. Genes Immun 10:647–653.
157. Limou S, Melica G, Coulonges C, Lelievre J-D, Do H, McGinn S, G. Gut I, Levy Y, Zagury J-F. 2012. Identification of IL7RA Risk Alleles for Rapid Progression During HIV-1 Infection: A Comprehensive Study in the GRIV Cohort. Curr HIV Res 10:143–150.
158. Guzmán-Fulgencio M, Berenguer J, Jiménez-Sousa MA, Pineda-Tenor D, Aldámiz-Echevarria T, García-Broncano P, Carrero A, García-Álvarez M, Tejerina F, Diez C, Vazquez-Morón S, Resino S. 2015. Association between IL7R polymorphisms and severe liver disease in HIV/HCV coinfected patients: a cross-sectional study. J Transl Med 13:206.
159. Andersson Å, Srivastava MK, Harris-White M, Huang M, Zhu L, Elashoff D, Strieter RM, Dubinett SM, Sharma S. 2011. Role of CXCR3 ligands in IL-7/IL-7Rα-Fc-mediated antitumor activity in lung cancer. Clin Cancer Res 17:3660–3672.
160. Vranjkovic A, Crawley AM, Gee K, Kumar A, Angel JB. 2007. IL-7 decreases IL-7 receptor α (CD127) expression and induces the shedding of CD127 by human CD8+ T cells. Int Immunol 19:1329–1339.
161. Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, Battaglia CA, Landay AL, Pahwa S, Fischl MA, Asmuth DM, Tenorio AR, Altman JD, Fox L, Moir S, Malaspina A, Morre M, Buffet R, Silvestri G, Lederman MM. 2009. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. Blood 113:6304–6314.
162. Ménétrier-Caux C, Ray-Coquard I, Blay JY, Caux C. 2019. Lymphopenia in Cancer Patients and its Effects on Response to Immunotherapy: An opportunity for combination with Cytokines? J Immunother Cancer. BioMed Central Ltd.
163. Sportès C, Babb RR, Krumlauf MC, Hakim FT, Steinberg SM, Chow CK, Brown MR, Fleisher TA, Noel P, Maric I, Stetler-Stevenson M, Engel J, Buffet R, Morre M, Amato RJ, Pecora A, Mackall CL, Gress RE. 2010. Phase I study of recombinant human interleukin-7 administration in subjects with refractory malignancy. Clin Cancer Res 16:727–735.

164. Surh CD, Sprent J. 2008. Homeostasis of Naive and Memory T Cells. Immunity 29:848–862.
165. Sprent J, Surh CD. 2011. Normal T cell homeostasis: The conversion of naive cells into memory-phenotype cells. Nat Immunol.
166. Leone A, Rohankhedkar M, Okoye A, Legasse A, Axthelm MK, Villinger F, Piatak M, Lifson JD, Assouline B, Morre M, Picker LJ, Sodora DL. 2010. Increased CD4 + T Cell Levels during IL-7 Administration of Antiretroviral Therapy-Treated Simian Immunodeficiency Virus-Positive Macaques Are Not Dependent on Strong Proliferative Responses . J Immunol 185:1650–1659.
167. Sportès C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, Fleisher TA, Krumlauf MC, Babb RR, Chow CK, Fry TJ, Engels J, Buffet R, Morre M, Amato RJ, Venzon DJ, Korngold R, Pecora A, Gress RE, Mackall CL. 2008. Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. J Exp Med 205:1701–1714.
168. Geiselhart LA, Humphries CA, Theresa A, Mou S, Subleski J, Kristin L, Geiselhart LA, Humphries CA, Gregorio TA, Mou S, Subleski J, Komschlies KL. 2001. IL-7 Administration Alters the CD4:CD8 Ratio, Increases T Cell Numbers, and Increases T Cell Function in the Absence of Activation. J Immunol 166:3019–3027.
169. Komschlies KL, Gregorio TA, Gruys ME, Back TC, Faltynek CR, Wiltrout RH. 1994. Administration of recombinant human IL-7 to mice alters the composition of B-lineage cells and T cell subsets, enhances T cell function, and induces regression of established metastases. J Immunol 152:5776–5784.
170. Guimond M, Veenstra RG, Grindler DJ, Zhang H, Cui Y, Murphy RD, Kim SY, Na R, Hennighausen L, Kurtulus S, Erman B, Matzinger P, Merchant MS, Mackall CL. 2009. Interleukin 7 signaling in dendritic cells regulates the homeostatic proliferation and niche size of CD4+ T cells. Nat Immunol 10:149–157.
171. Foss DL, Donskoy E, Goldschneider I. 2001. The importation of hematogenous precursors by the thymus is a gated phenomenon in normal adult mice. J Exp Med 193:365–374.
172. Krueger A, Ziętara N, Łyszkiewicz M. 2017. T Cell Development by the Numbers. Trends Immunol. Elsevier Ltd.
173. Komschlies KL, Back TT, Gregorio TA, Gruys ME, Damia G, Wiltrout RH, Faltynek CR. 1994. Effects of rhIL-7 on leukocyte subsets in mice: implications for antitumor activity. Immunol Ser 61:95–104.
174. Geiselhart LA, Humphries CA, Gregorio TA, Mou S, Subleski J, Komschlies KL. 2001. IL-7 Administration Alters the CD4:CD8 Ratio, Increases T Cell Numbers, and Increases T Cell Function in the Absence of Activation. J Immunol 166:3019–3027.
175. Vieira M, Soares D, Borthwick NJ, Maini MK, Janossy G, Salmon M, Akbar AN. 1998. IL-7-Dependent Extrathymic Expansion of CD45RA⁺ T Cells Enables Preservation of a Naive Repertoire. J Immunol 161:5909 LP – 5917.
176. Moniuszko M, Fry TJ, Tsai W-P, Morre M, Assouline B, Cortez P, Lewis MG, Cairns S, Mackall CL, Franchini G. 2004. Recombinant interleukin-7 induces proliferation of naive macaque CD4+ and CD8+ T cells in vivo. J Virol 78:9740–9749.
177. Boonnak K, Vogel L, Feldmann F, Feldmann H, Legge KL, Subbarao K. 2014. Lymphopenia Associated with Highly Virulent H5N1 Virus Infection Due to Plasmacytoid Dendritic Cell–Mediated Apoptosis of T Cells. J Immunol 192:5906–5912.

178. Min B, Yamane H, Hu-Li J, Paul WE. 2005. Spontaneous and Homeostatic Proliferation of CD4 T Cells Are Regulated by Different Mechanisms. J Immunol 174:6039–6044.
179. Shindo Y, Fuchs AG, Davis CG, Eitas T, Unsinger J, Burnham C-AD, Green JM, Morre M, Bochicchio G V., Hotchkiss RS. 2017. Interleukin 7 immunotherapy improves host immunity and survival in a two-hit model of Pseudomonas aeruginosa pneumonia . J Leukoc Biol 101:543–554.
180. Von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SEG, Murray R. 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med 181:1519–1526.
181. Loubaki L, Tremblay T, Bazin R. 2013. In vivo depletion of leukocytes and platelets following injection of T cell-specific antibodies into mice. J Immunol Methods 393:38–44.
182. Jung SR, Suprunenko T, Ashhurst TM, King NJC, Hofer MJ. 2018. Collateral Damage: What Effect Does Anti-CD4 and Anti-CD8α Antibody–Mediated Depletion Have on Leukocyte Populations? J Immunol 201:2176–2186.
183. Grčević D, Lee S-K, Marušić A, Lorenzo JA. 2000. Depletion of CD4 and CD8 T Lymphocytes in Mice In Vivo Enhances 1,25-Dihydroxyvitamin D 3 -Stimulated Osteoclast-Like Cell Formation In Vitro by a Mechanism That Is Dependent on Prostaglandin Synthesis . J Immunol 165:4231–4238.
184. Engram JC, Cervasi B, Borghans JAM, Klatt NR, Gordon SN, Chahroudi A, Else JG, Mittler RS, Sodora DL, De Boer RJ, Brenchley JM, Silvestri G, Paiardini M. 2010. Lineage-specific T-cell reconstitution following in vivo CD4+ and CD8+ lymphocyte depletion in nonhuman primates. Blood.
185. Martínez C, Urbano-Ispizua A, Rovira M, Carreras E. 2000. Immune Reconstitution Following Allogeneic Peripheral Blood Progenitor Cell Transplantation. Ciril Rozman & Emilio Montserrat 37:535–542.
186. Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM, Magrath IT, Wexler LH, Dimitrov DS, Gress RE. 1997. Distinctions Between CD8+ and CD4+ T-Cell Regenerative Pathways Result in Prolonged T-Cell Subset Imbalance After Intensive Chemotherapy. Blood 89:3700–3707.
187. Mackall CL, Fry TJ, Bare C, Morgan P, Galbraith A, Gress RE. 2001. IL-7 increases both thymic-dependent and thymic-independent T-cell regeneration after bone marrow transplantation. Blood 97:1491–1498.
188. Politikos I, Boussiotis VA. 2014. The role of the thymus in T-cell immune reconstitution after umbilical cord blood transplantation. Blood. American Society of Hematology.
189. Mackall CL, Bare C V, Granger LA, Sharrow SO, Titus JA, Gress RE. 1996. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol 156:4609–16.
190. Llano A, Barretina J, Gutiérrez A, Blanco J, Cabrera C, Clotet B, Esté JA. 2001. Interleukin-7 in Plasma Correlates with CD4 T-Cell Depletion and May Be Associated with Emergence of Syncytium-Inducing Variants in Human Immunodeficiency Virus Type 1-Positive Individuals. J Virol 75:10319–10325.
191. Bolotin E, Annett G, Parkman R, Weinberg K. 1999. Serum levels of IL-7 in bone marrow transplant recipients: Relationship to clinical characteristics and lymphocyte count. Bone Marrow Transplant 23:783–788.

192. Broers AEC, Posthumus-Van Sluijs SJ, Spits H, Van der Holt B, Löwenberg B, Braakman E, Cornelissen JJ. 2003. Interleukin-7 improves T-cell recovery after experimental T-cell-depleted bone marrow transplantation in T-cell-deficient mice by strong expansion of recent thymic emigrants. Blood 102:1534–1540.
193. Waller EK, Giver CR, Rosenthal H, Somani J, Langston AA, Lonial S, Roback JD, Li JM, Hossain MS, Redei I. 2004. Facilitating T-cell immune reconstitution after haploidentical transplantation in adults, p. 233–237. *In* Blood Cells, Molecules, and Diseases.
194. Waller EK, Logan BR, Fei M, Lee SJ, Confer D, Howard A, Chandrakasan S, Anasetti C, Fernando SM, Giver CR. 2019. Kinetics of immune cell reconstitution predict survival in allogeneic bone marrow and G-CSF-mobilized stem cell transplantation. Blood Adv 3:2250–2263.
195. Male, David; Roitt, Ivan; Brostoff, Jonathan; Roth D. 2006. Immunology7th editio. Elsevier Health Sciences, 2006.

7. Appendix

7.1 Rights and Permission

Permission to use from publisher group:

Obtain permission request - Book (1015942); photo res
Submission ID: 1015942
Date: 20 May 2020 12:49am
Name: Master student Nawaf Aloufi
Institute/company: University of Ottawa
Type of Publication: Book
Title: Immunology, 7th Edition
ISBN: 9780323047821
Auhtors: David Male, Jonathan Brostoff, David Roth, Ivan Roitt, and David Male
Year: 2006
From page: 39
To page: 39
Chapter number: 2
Chapter title: Cells, tissues and organs of the immune system
I would like to use: Figure(s)
Quantity of material: 1 figure: Figure 2.26
I am the author of the Elsevier material: No
Elsevier author is involved in my project: No
In what format will you use the material: Print and Electronic
Translation: No
Proposed use: Reuse in a thesis/dissertation
Additional Comments / Information: I would like to get permission to reuse the figure ASAP, as my deadline for submitting my thesis is in a week from now.

Dear Nawaf Aloufi

We hereby grant you permission to reprint the aforementioned material at no charge **in your thesis, in print and on the University web site** subject to the following conditions:

6. If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies.

2. Suitable acknowledgment to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"This article was published in Publication title, Vol number, Author(s), Title of article, Page Nos, Copyright Elsevier (or appropriate Society name) (Year)."

3. Your thesis may be submitted to your institution in either print or electronic form.

4. Reproduction of this material is confined to the purpose for which permission is hereby given.

5. This permission is granted for non-exclusive world **English** rights only. For other languages please reapply separately for each one required. Permission excludes use in an electronic form other than as specified above. Should you have a specific electronic project in mind please reapply for permission.

6. Should your thesis be published commercially, please reapply for permission.

Kind Regards
Anita

Anita Mercy
Senior Copyrights Coordinator – Copyrights Team
Elsevier | Health Content Operations
(A division of Reed Elsevier India Pvt. Ltd.)
Ascendas International Tech Park, Crest – 12th Floor|Taramani, Chennai 600113 • India|
Tel: +91 4442994696
a.vethakkan@elsevier.com

Permissions Helpdesk please visit – Permissions Helpdesk Support Center

Figure 1-2 Permission to use from publisher group:

SPRINGER NATURE LICENSE TERMS AND CONDITIONS

May 19, 2020

This Agreement between University of Ottawa -- Nawaf Aloufi ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4750931461624
License date	Jan 16, 2020
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Nature Reviews Immunology
Licensed Content Title	Harnessing the biology of IL-7 for therapeutic application
Licensed Content Author	Crystal L. Mackall et al
Licensed Content Date	Apr 21, 2011
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	figures/tables/illustrations
Number of figures/tables/illustrations	1
High-res required	no
Will you be translating?	no
Circulation/distribution	1 - 29
Author of this Springer Nature content	no
Title	The role of sCD127 in mediating IL-7 function on T-cell homeostasis in vivo
Institution name	University of Ottawa
Expected presentation date	Mar 2020
Portions	Figure 2
Requestor Location	University of Ottawa 75 Laurier Ave Ottawa, ON K1N 6N5 Canada Attn: University of Ottawa
Total	**0.00 CAD**
Terms and Conditions	

Springer Nature Customer Service Centre GmbH
Terms and Conditions

This agreement sets out the terms and conditions of the licence (the **Licence**) between you and **Springer Nature Customer Service Centre GmbH** (the **Licensor**). By clicking 'accept' and completing the transaction for the material (**Licensed Material**), you also confirm your acceptance of these terms and conditions.

1. Grant of License

1. 1. The Licensor grants you a personal, non-exclusive, non-transferable, world-wide licence to reproduce the Licensed Material for the purpose specified in your order only. Licences are granted for the specific use requested in the order and for no other use, subject to the conditions below.

1. 2. The Licensor warrants that it has, to the best of its knowledge, the rights to license reuse of the Licensed Material. However, you should ensure that the material you are requesting is original to the Licensor and does not carry the

copyright of another entity (as credited in the published version).

1. 3. If the credit line on any part of the material you have requested indicates that it was reprinted or adapted with permission from another source, then you should also seek permission from that source to reuse the material.

2. Scope of Licence

2. 1. You may only use the Licensed Content in the manner and to the extent permitted by these Ts&Cs and any applicable laws.

2. 2. A separate licence may be required for any additional use of the Licensed Material, e.g. where a licence has been purchased for print only use, separate permission must be obtained for electronic re-use. Similarly, a licence is only valid in the language selected and does not apply for editions in other languages unless additional translation rights have been granted separately in the licence. Any content owned by third parties are expressly excluded from the licence.

2. 3. Similarly, rights for additional components such as custom editions and derivatives require additional permission and may be subject to an additional fee. Please apply to Journalpermissions@springernature.com/bookpermissions@springernature.com for these rights.

2. 4. Where permission has been granted **free of charge** for material in print, permission may also be granted for any electronic version of that work, provided that the material is incidental to your work as a whole and that the electronic version is essentially equivalent to, or substitutes for, the print version.

2. 5. An alternative scope of licence may apply to signatories of the STM Permissions Guidelines, as amended from time to time.

3. Duration of Licence

3. 1. A licence for is valid from the date of purchase ('Licence Date') at the end of the relevant period in the below table:

Scope of Licence	Duration of Licence
Post on a website	12 months
Presentations	12 months
Books and journals	Lifetime of the edition in the language purchased

4. Acknowledgement

4. 1. The Licensor's permission must be acknowledged next to the Licenced Material in print. In electronic form, this acknowledgement must be visible at the same time as the figures/tables/illustrations or abstract, and must be hyperlinked to the journal/book's homepage. Our required acknowledgement format is in the Appendix below.

5. Restrictions on use

5. 1. Use of the Licensed Material may be permitted for incidental promotional use and minor editing privileges e.g. minor adaptations of single figures, changes of format, colour and/or style where the adaptation is credited as set out in Appendix 1 below. Any other changes including but not limited to, cropping, adapting, omitting material that affect the meaning, intention or moral rights of the author are strictly prohibited.

5. 2. You must not use any Licensed Material as part of any design or trademark.

5. 3. Licensed Material may be used in Open Access Publications (OAP) before publication by Springer Nature, but any Licensed Material must be removed from OAP sites prior to final publication.

6. Ownership of Rights

6. 1. Licensed Material remains the property of either Licensor or the relevant third party and any rights not explicitly granted herein are expressly reserved.

7.2 Appendix Table

Table 7-1: Flow cytometry panels used to determine sCD127 and IL-7 activity on T cells.

	FL1 (FITC) Blue Laser	FL2 (PE) Yellow Green Laser	FL5 (PE-Cy7) Yellow Green Laser	FL6 (V450) Violet Laser	FL7 (BV510) Violet Laser	FL9 (BV786) Violet Laser	FL9 (APC) Red Laser
T cell Proliferation and Proportion Assessment							
SET 1	CD3	NK1.1		Ki-67	Dead	CD4	CD8
SET 2	CD3	NK1.1		~~Ki-67~~ FMO	Dead	CD4	CD8
Membrane CD127 expression on T cells							
SET 3	CD3	CD127			Dead	CD4	CD8
SET 5	CD3	CD127 ISO CN			Dead	CD4	CD8
T cell Phenotype Assessment							
SET 6	CD62L	CD44	CD8	TCRβ	Dead	CD4	
SET 7	CD62L ISO CN	CD44 ISO CN	CD8	TCRβ	Dead	CD4	

FMO: Fluorescence Minus One; ISO CN: Isotype Controls.

7.3 Appendix Figures:

Figure 7.1

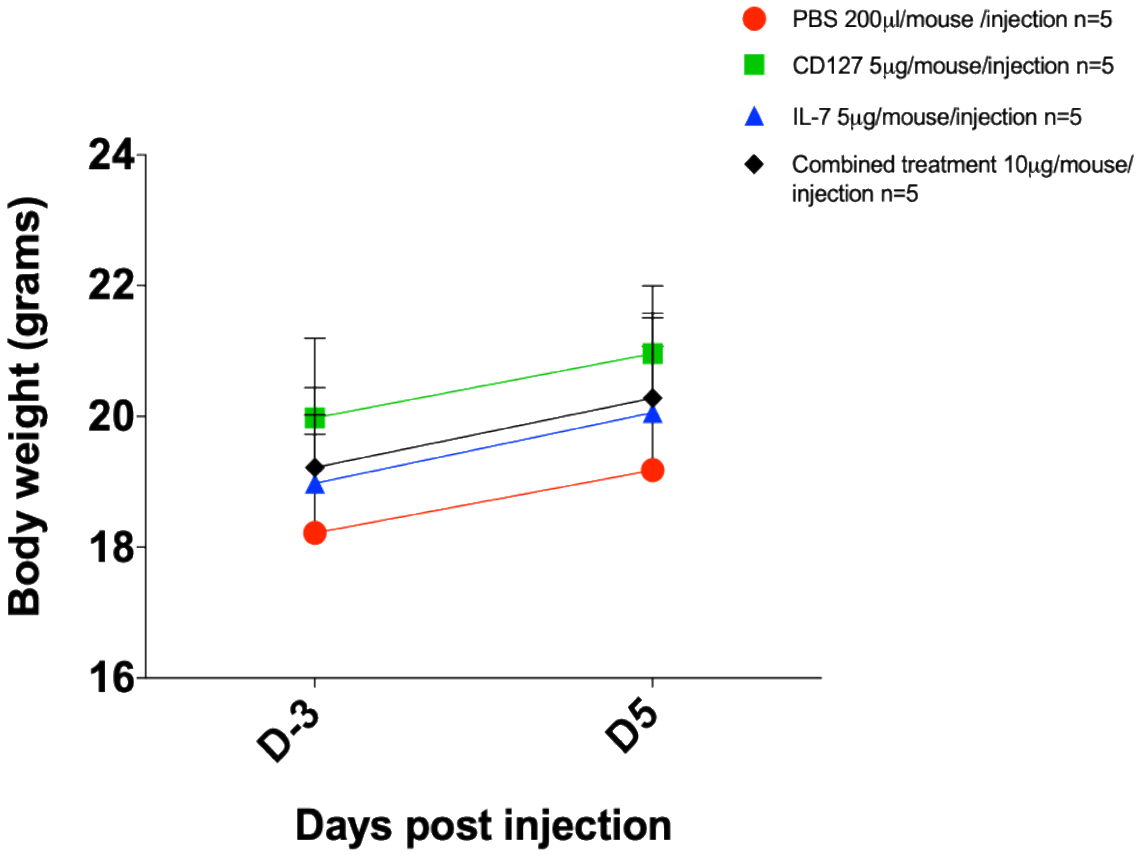


Figure 7-1: The effect of sCD127 and IL-7 treatments on mouse body weight, total n=20 mice. Body weight (in grams) of mice in each treatment group was recorded at baseline (d-3) and after treatment (d5) with sCD127, IL-7, or sCD127 complexed with IL-7, or PBS alone. The data presented here represent the mean body weight of mice in each treatment group ± SD.

8. Curriculum Vitae

Nawaf Abdulrahman Aloufi, MSc

EDUCATION

Master of Science: Microbiology and Immunology **2016 – 2020**
University of Ottawa
Ottawa, ON, Canada

- Thesis entitled: The role of sCD127 in IL-7-mediated T cell homeostasis *in vivo*.

Bachelor of Science: Laboratory Medicine Major **2009 – 2013**
Umm Alqura University
Mecca, Saudi Arabia

- Graduated with Excellent grade, GPA 3.74 out of 4.00
- Thesis entitled: Sero-epidemiological survey on rift valley fever among small ruminants and their close contact people in Makkah, Saudi Arabia.

WORK EXPERIENCE

MSc trainee **2016 – 2020**
Dr. Jonathan Angel laboratory
Microbiology and Immunology department
University of Ottawa
Ottawa, ON, Canada

- Trained on Data Analysis, Tissue Culture, Flow Cytometry, and other Immunoassay Techniques

Clinical laboratory Specialist in the Microbiology Lab **2013 –2014**
Department of Pathology and Laboratory Medicine
King Khalid National Guard Hospital
Jeddah, Saudi Arabia

- Worked in all routine diagnostic microbiology
- Worked on many automated specialized machines including VITEK II, BACTEALERT, and AUTION HYBRID

Medical Laboratory Trainee **2012 – 2013**
Department of Pathology and Laboratory Medicine
King Khalid National Guard Hospital
Jeddah, Saudi Arabia

- Worked in all Laboratories; Clinical Chemistry, Molecular Biology, Microbiology, Histopathology, Serology, Hematology, Blood Bank
- Acquired skills and gained experience in different automated analyzers and manual techniques in different laboratories

---

RESEARCH ACTIVITIES

- Nawaf Aloufi, Alaa Ali, Seung-Hwan Lee, Jonathan Angel. The Role of Soluble IL-7 Receptor α (sCD127) in IL-7-mediated T-cell Homeostasis *in vivo*. University of Ottawa Department of Biochemistry and Microbiology and Immunology: The BMI scientific symposium; Montebello, QC. **May 2018**. (Poster Presentation)
- Nawaf Aloufi, Alaa Ali, Sandra C. Côté, Seung-Hwan Lee, Jonathan Angel. The Role of sCD127 in IL-7-mediated T-cell Homeostasis *in vivo*. The 27th Annual Canadian Conference on HIV/AIDS Research; Vancouver, BC. **April 2018**. (Poster Presentation)
- Nawaf Aloufi, Seung-Hwan Lee, Jonathan Angel. The Role of sCD127 in IL-7-mediated T-cell Homeostasis *in vivo*. The Ottawa Hospital Research Institute's 17th annual Research Day; Ottawa, ON. **November 2017**. (Poster Presentation)

EXTRACURRICULAR ACTIVITIES

- **2015: Event Volunteer**, Saudi Students Society of British Colombia, Vancouver, BC, Canada
- **2013: Event Volunteer**, World Diabetes Day, National Guard Hospital, Jeddah, Saudi Arabia
- **2012: Organizer**, The first Scientific meeting for students' achievements, Laboratory Medicine department, Umm Alqura University
- **2012: Event Volunteer**, Awareness campaign about obesity in public areas, Mecca, Saudi Arabia

---

SYMPOSIA AND WORKSHOPS

- **2017:** Attended "**The 26th Annual Canadian Conference on HIV / AIDS Research"** Montreal, QC, Canada.
- **2017:** Attended **"The Symposium on Inflammation in Health and Disease"** at University of Ottawa, Ottawa, ON, Canada
- **2017:** Attended **"The 6th annual symposium on Cytokines in Inflammation, Ageing, Cancer and Obesity"** Eastman, QC, Canada
- **2013:** Attended **"Research Methodology workshop"** at King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

- **2012:** Attended the annual clinical and molecular microbiology laboratories activities entitled **"UPDATES IN MICROBIOLOGY 2012"** at King Abdulaziz University Hospital, Jeddah, Saudi Arabia.
- **2010:** Attended **"Microbiology and Communicable Diseases Conference"** at King Abdullah Medical City, Makkah, Saudi Arabia.

AWARDS & SCHOLARSHIPS

- **2016: Masters scholarship,** Ministry of Education, Saudi Arabia
- **2013: First place award - oral presentation,** Faculty of Applied Medical Science, Umm Alqura University
- **2012: President's (Vice-Chancellor) award for academic excellence,** Umm Alqura University

REFERENCES

Dr. Jonathan Angel (MD, FRCPC)
Head, Division of Infectious Diseases, Ottawa Hospital
Professor of Medicine, University of Ottawa
Senior Scientist, Ottawa Health Research Institute

Dr. Seung-Hwan Lee, PhD
Associate Professor
Department of Biochemistry, Microbiology and Immunology
University of Ottawa

Prof. Mohammad M. Khan, PhD
Professor of Microbiology
Laboratory Medicine Department
Faculty of Applied Medical Sciences
Umm Alqura University