ASSESSING THE EFFICACY OF INTERLEUKIN-7 AS AN

IMMUNOTHERAPEUTIC IN THE SIV+ RHESUS MACAQUE MODEL

APPROVED BY SUPERVISORY COMMITTEE

Dr. Donald Sodora Ph.D

Dr. Iwona Stroynowski Ph.D.

Dr. David Farrar Ph.D.

Dr. Julie Pfeiffer Ph.D.

Dr. Vanessa Sperandio Ph.D.

DEDICATION

I would like to thank my mentors Dr. Donald Sodora and Dr. Louis Picker for their continuing support and invaluable guidance over the course of my graduate experience. The dedication to scientific curiosity and intellectual rigor they modeled made my graduate experience much richer than words can express.

I would also like to thank my graduate committee who was called on in unexpected ways for guidance and support. Their critiques, questions, comments and support were always insightful and always appreciated.

ASSESSING THE EFFICACY OF INTERLEUKIN-7 AS AN IMMUNOTHERAPEUTIC IN THE SIV+ RHESUS MACAQUE MODEL

by

AMANDA KATHLEEN LEONE

DISSERTATION

Presented to the Faculty of the Graduate School of Biomedical Sciences

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

The University of Texas Southwestern Medical Center at Dallas

Dallas, Texas

October 28, 2009

ASSESSING THE EFFICACY OF INTERLEUKIN-7 AS AN IMMUNOTHERAPEUTIC IN THE SIV+ RHESUS MACAQUE MODEL

Publication No. _____

Amanda Kathleen Leone, Ph.D.

The University of Texas Southwestern Medical Center at Dallas, 2009

Supervising Professor: Dr. Donald Sodora Ph.D.

Human Immunodeficiency Virus (HIV) infection is known for depleting 'helper' CD4+ T cells. Highly active antiretroviral therapy (HAART) reduces viremia and increases CD4+ levels, however, 5-20% of patients fail to reconstitute CD4+ T cell levels despite viral suppression. Interluekin-7 (IL-7), a homeostatic cytokine, increases proliferation and survival of memory T cells. It is also a candidate immune therapeutic to assist CD4+ T cell recovery following HIV infection. Simian immunodeficiency virus (SIV) infection of Rhesus macaques, mimics the disease course of HIV patients and has been used to study HIV pathogenesis and treatment. The goal of this dissertation was to identify a strategy for administering IL-7 to SIV+ anti-retroviral therapy (ART) treated macaques to increase CD4+ T cell levels long-term. Glycosylated recombinant

macaque IL-7 was given subcutaneously at 7 day to 6-week intervals. Proliferation, and levels, of naïve and memory CD4+ T cells, as well as other immune cell subsets were assessed. Irrespective of the dosing interval tested IL-7 transiently increased proliferation of memory and naïve cells, in CD4+ and CD8+ subsets without increasing plasma SIV titers. CD4+ T cells proliferated following each IL-7 administration at 6-week intervals, but absolute levels increased only transiently. In contrast, a frequent IL-7 dosing regimen (weekly x 3, with 2 weeks rest repeated twice) induced a single proliferative burst in CD4+ T cells but T cell levels were increased >112 days post IL-7 treatment. This strategy also increased the half-life of bromodeoxy-uridine (BrDU) labeled memory T cells in the blood when compared to ART alone, consistent with enhanced cell survival. Further, we show that untreated SIV+ macaques have attenuated proliferation compared to uninfected macaques (and ART treated macaques) with minimally increased T cell levels following IL-7. Additionally, chronic SIV infection is associated with impaired STAT5 activation, which may possibly decrease cell survival. These data suggest that administering IL-7 at frequent intervals in conjunction with ART is the optimal strategy to obtain sustained increases of memory CD4+ T cell levels. These findings in the SIV-macaque model provide evidence that IL-7 is a potentially broad acting immune therapeutic that could be administered to HIV+ patients that do not fully restore CD4+ T cell levels after HAART treatment.

Table of Contents

PRIOR PUBLICATIONS

- **Leone, A**; Rohankhedkhar, M; Okoye, A; Legasse, A; Axthelm, M, Villinger, F; Piatak, M; Lifson, J; Assouline, B; Moore, M; Picker, LJ and Sodora, DL. *Assessing the Immunotherapeutic Potential of Interleukin-7 in ART treated SIV+ Macaques.* Submitted to Journal of Immunology
- Kuhrt, D; Faith, S; **Leone, AK**; Rohankhedkhar, M; Sodora, D; Picker, LJ; and Stefano-Cole, K. *Simian Immunodeficiency Virus Results in Rapid Depletino of Selected B Cell subsets from the Periphery during Acute Infection.* Submitted J. Virology
- **Leone, A;** Picker, LJ and Sodora, DL. *IL-2, Il-7 and IL-15 as Immunomodulators during SIV/HIV Vaccination and Treatment.* Curr HIV Res. 2009 Jan; 7(1): 83-90
- David Kosub, Ginger Lehrmann, Mamta Jain, Jeffrey Milush, Dejiang Zhou, Elizabeth Chacko, **Amanda K Leone**, Philip Keiser, Guido Silvestri and Donald Sodora. *Gamma/Delta T-cell Functional Responses differ after Pathogenic Human Immunodeficiency Virus and Non-pathogenic Simian Immunodeficiency Virus Infections.* J. Virol 2008 Feb; 82(3): 1155-62 Epub 2007 Nov 28
- **Leone, AK;** Chun, JA, Caranto, J; Scudder, JD; and King, JM. *Epithelial Barrier Function and Matrix-Metalloproteinase 9 Activity are regulated by Tumor Necrosis Factor-a and Interferon-g in Madin-Darby Canine Kidney Cells.* Cell Physiol and Biochem 2007; 19 (1-4): 99-112
- Patrick, DM; **Leone, AK**; Shellenberger, JJ; Dudowicz, KA; and King, JM. *Proinflammatory Cytokines Tumor Necrosis Factor-a and Interferon-g Modulate Epithelial Barrier Function in Madin-Darby Canine Kidney Cells through Mitogen Activated Protein Kinase Signaling.* BMC Physiology 2006 6:2

LIST OF FIGURES

LIST OF TABLES

LIST OF DEFINITIONS

Ag- Antigen

AIDS – Acquired Immunodeficiency Syndrome

APC- Antigen presenting cell

ART- Antiretroviral therapy; PMPA/FTC treatment in Rhesus macaques

BrdU- 5-bromo-2'-deoxyuridine

CM- Central memory T cells ($CD28^+$, $CD95^+$, $CCR5^-$, $CCR7^+$)

EM- Effector Memory T cells ($CD28^-$, $CD95^+$, $CCR5^+$, $CCR7^-$)

FDA- Federal Drug Administration

FTC- Nucleoside analog used in ART in macaques

GVHD- Graft versus host disease

HAART- Highly Active Antiretroviral Therapy; combination therapy in patients

HIV – Human Immunodeficiency Virus

HSCT- Hematopoietic Stem Cell Transfer

IL-2 – Interleukin-2

IL-2R(a)- Interleukin-2 receptor; CD25

IL-7 – Interleukin-7

IL-7R(a)- Interleukin-7 receptor; CD127

IL-15 – Interleukin-15

IL-15R(a)- Interleukin-15 receptor;

Ki67- Nuclear antigen used as a marker for cells entering S phase within a 4-7 period

JAK – Janus Kinase (3)

Macaque- Rhesus macaque (*Macaca mulatta)*

MAPK- Mitogen Activated Protein Kinase

ONPRC- Oregon National Primate Center

OI- Opportunistic Infection

Naïve/N- Naïve T cells ($CD28^{moderate}$, $CD95^{-}$, $CCR5^{-}$, $CCR7^{moderate}$)

NHP- Non-human primate

NK- Natural Killer Cells

PBMCs- Peripheral blood mononuclear cells

PI3K – Phosphoinositde-3-Kinase

PMPA- 9-R-2-Phosphonomethoxypropyl adenine; nucleoside analog used in macaques

rhIL-7- Recombinant human IL-7 non-glycosylated

rmIL-7/rsIL-7 –Recombinant macaque IL-7 non-glycosylated

rsIL-7gly- Glycosylated recombinant macaque IL-7 produced by Cytheris Inc.

sAIDS- Simian AIDS

SHIV- Recombinant virus expressing SIV envelope proteins in an HIV backbone

SIV-Simian Immunodeficiency Virus

SIVmac251- Pathogenic SIV variant quasi species containing multiple SIV strains

SIVmac239- Pathogenic SIV strain derived from SIVmac251 containing a single clone

STAT – Signal Transducer and Activators of Transcription (5)

TCR- T cell receptor

Treg – CD4+CD25+ regulatory T cells

TrM- Transitional memory T cells ($CD28^{+,}$, $CD95^{+}$, $CCR5^{+}$)

CHAPTER ONE

INTRODUCTION

Basic Principles and General Context:

Acquired Immunodeficiency Syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) and is one of the leading causes of morbidity and mortality, recent reports estimate between 30-60 million infections worldwide (14, 129, 232, 234) and as much as one quarter of the population in sub-Saharan Africa is infected (234). HIV-1 can be subdivided into three broad classes the M, N and O strains (129). The M (main) group is responsible for the HIV pandemic. Multiple clades exist within the M group including subtype B, the dominant clade in Europe, North America and Australia and subtypes A and C that are more common in Africa (129).

The extensive variation in HIV strains leads to several challenges for vaccine development. In response to a recent vaccine failure (Merck's STEP trial) the field recently worked together to collaboratively define the challenges to vaccine development which include: 1) the extensive genetic diversity in HIV subtypes, 2) early establishment of latent reservoirs, 3) incomplete knowledge of what constitutes immunological protection from HIV infection, 4) efficient immune evasion strategies by the virus, 5) no current method to reliably elicit neutralizing

antibodies effective against a broad range of clades and 6) incomplete understanding of events leading to transmission across a mucosal barrier [reviewed by (14, 102). The most promising leads toward developing a vaccine thus far come from the recently completed Thai trails (231), which used a prime-boost strategy consisting of four 'priming' injections using a recombinant canarypox vector and boosted with purified HIV envelope protein, exhibited some protective effects. However, a truly effective protective HIV vaccine is unlikely in the near future and therefore to mitigate the human impact of HIV infection novel viral and immune therapeutic approaches are required.

HIV transmission generally occurs across mucosal barriers with relatively low frequency estimated to range from 1/10 to 1/600 coital acts in men who have sex with men up to 1/200 to 1/10,000 coital acts when transmission occurs from female to male (102). The lifecycle of the virus after infecting a target cell has been reviewed previously (8). Briefly, the HIV genome consists of two identical 9kb single stranded RNA molecules that encode a total of 9 proteins (257). HIV preferentially infects CD4+ T cells and macrophages by binding to the CD4 receptor via the envelope (Env) protein as well as a coreceptor, most commonly CCR5 or CXCR4 (82, 98, 99, 257). Env binding initiates a conformational change in the protein exposing key peptides (gp41) required to complete fusion between the virus and host cell membranes (98, 99). The viral core uncoats

releasing the RNA genome that is reverse transcribed to proviral DNA by the virally encoded reverse transcriptaseRT enzyme (82, 99, 257). The double stranded proviral DNA is actively transported into the nucleus as part of the preintegration complex where it is inserted into the chromosome by the integrase enzyme (99, 257). For integration to occur however, the target cell must be activated (82). Once inside the cell, HIV arrests the cell cycle to prolong the length of time that synthesis of new virions can occur using the viral proteins (e.g vpr) (302). HIV proteins are synthesized in one polyprotein that is cleaved by a virally encoded protease to form the components for new virions (257). There are currently three accepted models for budding with new virions being derived from lipid rafts, exported from the golgi in exosomes and/or spreading directly by cell to cell transfer through an infection synapse (98).

SIV/HIV Pathogenesis:

One of the defining features of SIV/HIV infection is the damage rendered to the host immune system. To fully appreciate the nuances of the damage inflicted by the virus it is first necessary to discuss the components of the immune system in its "normal" state. The immune system is an extremely heterogeneous population of cells related loosely by their role in fighting invading pathogens. B-cells and T-cells form the adaptive arm of the immune system. Interleukin-7 (IL-7) is one of the key regulators of development for both cell types (6, 83, 107, 116, 145,

163, 174, 198, 203). The role of IL-7 in B-cell development was originally identified in IL-7 deficient mice that showed a reduced number of mature B cells (218, 286). Surprisingly, clinical studies have demonstrated that patients with IL-7 receptor (IL-7R/CD127) deficiency have normal levels of B cells (95). In-vitro studies coculturing lymphoid progenitors from human cord blood or adult bone marrow with bone marrow stromal cell lines have suggested a model in which human fetal/neonatal B-cell development is IL-7 independent while adult B-cell development is IL-7 dependent (119, 215, 273). Conventional alpha/beta T cells develop in the thymus and then emigrate to the periphery carrying either the CD4+ or the CD8+ co receptor (96). IL-7 deficient mice and patients lacking a functional IL-7R display severe T cell lymphopenia demonstrating the importance of this cytokine in T cell development (163, 226, 227, 236). In the periphery, CD4+ and CD8+ T cells can be subdivided into multiple subsets on the basis of their phenotype and function. Antigen inexperienced T cells, cells that have not met their cognate antigen, are classified as naïve T cells (N). These cells express low levels of activation markers and circulate through secondary lymphoid tissue [reviewed by (246)]. Naïve cells exhibit the capacity to expand clonally upon exposure to specific antigens while differentiating into effector and memory T cells (246). This population expresses high levels of IL-7R and is maintained through a combination of new emigrants from the thymus (198, 266) and homeostasis, mediated in large part through synergistic signals downstream of the

T cell receptor and IL-7R (115, 245, 275). After exposure to a specific pathogen, naïve cells differentiate into memory cells, which can be further subdivided into central (CM) and effector memory (EM) subsets on the basis of function. One different between CM and EM subsets is their localization within the body, CM cells are found in secondary lymphoid tissue while EM cells are at mucosal sites, and their functional response to pathogens, CM cells are the major repository of immune memory and clonally expand differentiating into effector cells upon second exposure to antigen while EM cells have limited proliferative potential but readily produce large amounts of cytokine upon exposure to antigen [differentiation is reviewed eloquently by (246, 296)]. The IL-7R is absent from the majority of EM cells, however recent studies have proposed that CM CD4+ and CD8+ T cells retaining IL-7R expression during an antigen specific immune response go on to become long lived CM cells (161, 246). CM cells express high levels of IL-7R and are maintained in the periphery via CD127 signaling, without requiring additional TCR signals (34, 115, 161, 246). The studies that have yielded the data with the highest resolution thus far into the immune dysfunction in B and T cells caused by HIV infection have been conducted in the SIV/rhesus macaque model.

The SIV/macaque model is closely analogous to HIV infection in humans including high levels of viral replication, elevated levels of immune activation

(44, 75, 123, 258), rapid depletion of CCR5+ CD4+ cells at mucosal sites over weeks (36, 151, 169, 219) and gradual depletion of peripheral blood CD4+ T cells over the course of years (169, 219). In addition to similar clinical disease courses there is strong genetic evidence that HIV-1 and HIV-2 are the result of zoonotic transmission of SIV from naturally infected primates to humans (201, 274). Although powerful the macaque model is limited in key areas: 1) macaque studies often require years to conduct and are extremely expensive, 2) macaques are a genetically out bred population leading to increased variation between animals and 3) SIV while a close homolog is not HIV itself. The strengths of the macaque model lie in the ability to precisely control the course of the infection; rigorous sampling of infected tissues and a genetically out bred population that is more reflective of human populations than genetically inbred murine models.

Experiments in the macaque model have allowed us to describe with a higher degree of resolution the depletion of CD4+ T cells observed in HIV patients and SIV infected macaques and allowed us to hypothesis the mechanisms contributing to the decline. CD4+ EM memory cell populations that express CCR5 are the primary targets of HIV and SIV, with the CM population serving as the major source of EM generation (100). During the acute phase of SIV infection there is an immediate and precipitous depletion of EM cells at mucosal sites (151, 169, 202, 219, 283). However, in most cases, the macaque survives the initial

onslaught although rapid progression to AIDS and death has been documented (97, 104-106, 108, 142, 209, 219). Rapid progression has also been associated with higher levels of viral replication at peak viremia (146, 182) and failure to mount an effective antibody response during acute infection (146, 182). In the last five years work in the macaque model indicates that while overt immunosuppression is associated with destruction of the CD4+ EM compartment, the failure of this compartment can be traced to homeostatic failure of the CM population and inadequate EM production (208, 225, 285). Comparisons of proliferating T cells in the lymph nodes of rapid and slow progressing macaques has bolstered this hypothesis, demonstrating that rapid progressing macaques have a significantly fewer proliferating cells/mm3 in the T cell area of the lymph node than macaques with slow or normal progression that are able to maintain elevated Ki67 over several months (182). The number of apoptotic cells/mm3 in the rapid and slow progressing cohorts in this study were similar indicating that the proliferating T cells in slow progressors were likely surviving and contributing to the overall higher T cell levels (182). The stress on the CD4+ CM compartment is compounded by impaired thymic function (reduced thymopoiesis) and tissue niche disruption (fibrosis in the lymph nodes) resulting in decreased naïve CD4+ T cell output slowing the rate at which new CM cells can be generated (66-68, 244, 285). Immune activation as well as CD4+ EM depletion has been implicated in the progression to sAIDS (123, 208, 219, 225, 258). In this context,

immune activation is characterized by increased turnover of CD3+ T cells (44, 62, 75, 213, 238, 258), increased expression of cellular activation markers including: HLA-DR (146, 225), CD38+ (123, 146), CD95+ (285) and CD69 (225), a high percentage of Ki67+ cells expressing CTLA-4 indicating activation and arrest in G1 phase of cell cycle rather than proliferation (146) increased T cell apoptosis in the lymph nodes (75, 182, 258, 285) and production of aberrantly high levels of inflammatory cytokines [Reviewed by (3);(123, 136)]. The level of immune activation measured during HIV/SIV infection has become one of the best predictors of the rate of disease progression with higher levels of immune activation associating with rapid onset of AIDS (146). One of the hypotheses to explain what is driving immune activation, is that microbial translocation, either whole organisms or microbial by-products, across damaged epithelium in the intestine continually triggers the host immune system (35). Chronic immune activation increases the number of activated memory CD4+ cells with two potential consequences: 1) The depletion of the CM compartment through increased cell death and differentiation; and 2) The increase in the number of activated HIV target cells where efficient viral replication can occur. HIV/SIV ultimately kills cells through a combination of lysis stemming from infection and indirect killing from immune activation (8).

Multiple classes of anti-retroviral drugs are now available such that highly active antiretroviral therapy (HAART) regimens can effectively suppress HIV replication in a majority of infected patients. HAART drugs effectively target various stages of the viral lifecycle. There are four key stages of the virus lifecycle targeted by HAART drugs: 1) entry, 2) reverse transcription, 3) proviral integration and 4) protease activity. Current HAART it sufficient to reduce the rate of viral replication and the level of immune activation, thus slowing both the loss of HIV susceptible CD4+ T cells and facilitating some regeneration (73). The patients with the best long-term outcome following initiation of HAART exhibit a reduction of viral load and increased CD4+ T cell numbers (5). However, 5-20% of patients exhibit a discordant response to initiation of highly active anti-retroviral therapy (HAART) resulting in an effective suppression of viral replication but only minimal CD4+ T-cell recovery (4, 113, 167). These discordant patients are candidates for novel immuno therapies.

Introduction to common γc-cytokines:

Current strategies for both replenishing diminished CD4+ memory T cell populations and for augmenting vaccine-induced anti-HIV immune responses have focused on cytokines that regulate proliferation, survival, activation and differentiation of T cells. Three cytokines of the common gamma-chain cytokine family, interleukin-2 (IL-2), interleukin-7 (IL-7) and interleukin-15 (IL-15) have

emerged as central regulators of peripheral T cell populations by virtue of their ability to induce proliferation and promote survival of particular T cell subsets as reviewed extensively elsewhere (20, 161, 246). These three cytokines share a common gamma-chain receptor CD132 and downstream signaling pathways leading to similar, but not identical, signaling via activation of the JAK/STAT and PI3K pathways. The canonical receptor consists of a high affinity ligand binding alpha chain and one or more signaling components including the common gamma chain. The IL-2R is a heterotrimer composed of the IL-2 binding alpha chain (CD25), the IL-2/IL-15R beta chain (CD122) and the common gamma chain (CD132) (11, 33, 161, 246). Much like the IL-2R, the IL-15 receptor (IL-15R) is a heterotrimer composed of a high affinity alpha chain (IL-15Ra), CD122 and CD132 (33, 161, 246). The IL-7 receptor (IL-7R) is a dimeric complex composed of a high affinity chain CD127 and CD132 (33, 161, 246). Activation of the JAK/STAT pathway downstream of the gamma chain receptor is associated with induction of anti-apoptotic proteins in the Bcl-2 family (12, 20, 116, 125, 294), while signaling in the PI3K pathway has a role in T cell proliferation (12, 20, 126, 128, 152). Although these cytokines share similar signaling pathways and have some overlapping functional effects there are distinct non-redundant effects due to: 1) Unique signaling events; 2) Different receptor expression patterns on T cell subsets; and 3) Differential expression of the cytokines in different tissues.

IL-2 was first of these cytokines to be discovered, and for many years was considered the major "T cell growth factor." While this T cell-derived cytokine can certainly support the expansion of Ag-stimulated CD4+ and CD8+ T cells, its primary non-redundant in vivo role in immune regulation is with regard to regulatory T cells (Tregs) and maintenance of peripheral tolerance (252, 279, 297). IL-2 is secreted by activated CD4+ T cells to induce proliferation of CD4+ T cells and to increase CD8+ T cell cytolytic potential (33, 161, 246). IL-2Ra (CD25) expression is also inducible following exposure to gamma chain cytokines including, IL-2, IL-7 and IL-15 or antigen (112, 159, 297). This creates a dual layer of regulation whereby the activity of IL-2 is controlled both by the availability of the cytokine and the receptor. IL-2 has been considered as a possible therapeutic for HIV for nearly two decades. It is the only one of the gamma chain cytokines currently approved for use in discordant HAART patients in Europe, the FDA has not approved it for use in HIV+ patients in the United States, but it has been approved for treating metastatic melanoma (195, 252).

IL-7 is crucial to the development and maintenance of mature conventional T cells. It has been shown to play a crucial role in promoting the expansion and maintenance of both thymic and peripheral T cell populations, including both the CD4+ and CD8+ lineages (145, 236, 286). Although many investigators have provided evidence that IL-7 is important for CD4+ T cell homeostasis during

HIV/SIV infection (89-92, 191, 192, 196), its exact role has not been fully elucidated. IL-7 is unique among the gamma chain cytokines in that it is constitutively produced in stromal cells of lymphoid tissues, rather than induced by exposure to a stimulus (197). One current hypothesis for IL-7 regulation suggests that decreases in T cell levels result in increased bioavailability of IL-7 per cell promoting survival and expansion in the remaining cells (34). At fully replete T cell levels, IL-7 availability is thought to be limiting thus promoting competition among cells expressing the IL-7Ra chain (34). This hypothesis is complicated by the findings of a recent study by Vranjkovic et al (287) indicating that CD127 is shed from the surface of CD8+ T cells following exposure to the cytokine.

While a primary role for IL-15 in-vivo is to expand the CD4+ and CD8+ EM T cells, it also has non-redundant roles in the growth and survival of NK cells and NK-T cells (19, 38, 187, 240). Evidence suggests that IL-15 provides both a proliferative and differentiation signal in T cells (157, 170, 189, 190, 220) such that responding CM cells not only proliferate, but also phenotypically and functionally differentiate into EM cells. IL-15 can mediate its physiological function through a membrane bound isoform rather than exclusively through a secreted form like IL-2 and IL-7 (70, 229) and is expressed in a wide variety of cell types (229, 243, 256). Therefore, an important consideration in using IL-15

as an immunotherapy is that the cytokine is often presented in trans to responsive cells enabling it to utilize juxtacrine signalling between IL-15 on one cell and IL-15R on another (19, 38, 70, 158, 176, 187, 239). It has been suggested that the IL-15 is able to signal through three receptor permutations; the canonical high affinity trimeric complex, an intermediate affinity dimeric receptor (170, 268) and signaling through the IL-15Ra chain (37, 40). The complicated nature of IL-15Ra is further realized in its ability to shed from cells whereby the soluble form of IL-15Ra potentially functions as a sink to sequester the active cytokine (38, 39, 187). The complexity of the IL-15/IL-15R interaction complicates the ability to develop IL-15 as an immune therapy to recover the HIV/SIV mediated damage to the host immune system.

Use of γc-cytokines during Non-HIV associated lymphopenia:

In primates, non-HIV associated anti-tumor models and total body irradiation during lymphoma treatments have provided a comprehensive model system for studying the role of gamma chain cytokines during the reconstitution of the human immune system following hematopoietic stem cell transplant (HSCT). The extensive lymphocyte depletion observed following radiation therapy (to prepare patients for bone marrow transplants) includes the dramatic loss of total T cells in a wide variety of tissues, including the thymus, and in this regard is similar to HIV/SIV infection (21, 262, 293). The two conditions differ in key

ways, unlike HIV/SIV infection, following radiation therapy both CD4+ and CD8+ T cells are equally depleted (21, 100, 143, 262, 293). In addition, radiation therapy leads to substantial loss of naïve T cells in addition to memory T cells, however the chronic phase of HIV infection most often preferentially depletes memory CD4+ T cells (21, 69, 100, 143, 210, 262, 293). Following HSCT, the depleted T cell compartment is replenished by two mechanisms, thymus dependent naïve cell production and thymic independent proliferation in the periphery (96, 262). Immediately following HSCT memory CD4+ and CD8+ T cells proliferate and the T cell compartment exhibits a T cell receptor repertoire limited by the available TCRs in the memory pool (77). Naïve cells do not appear in the peripheral blood for approximately 200 days post transplant and only after naïve cells begin to be produced by the HSCT recipient does the T cell repertoire expand (72, 76, 77). IL-7 has been used in clinical studies and has been shown in melanoma patients to expand memory and naive CD4+ and CD8+ T cells at similar rates (237). These results establish the potential for gamma chain cytokines to be used safely and effectively in lympho-depleted patients to aid in the reconstitution of the immune.

Use of γc-cytokines During SIV/HIV Infection:

HIV patients are confronted with ongoing viral replication, including low-level replication when on HAART, and chronically high levels of immune activation

(5, 103, 167). To be a viable therapy option for HIV patients, IL-2, IL-7 and IL-15 must demonstrate limited virological effects; and significant immunological benefit. The use of each gamma chain cytokine in the SIV+ macaque model, as well as human clinical trials, are reviewed below to assess both virologic and immunologic consequences of each cytokine.

Interleukin-2:

In patients, IL-2 has been used in two primary types of clinical trials, early studies using frequent high dose intravenous administration of the cytokine and more recent studies using infrequent low dose subcutaneous administration (60, 61, 80, 114, 137, 138, 143, 153, 166, 177, 252). In general, the patients enrolled in these studies are on stable HAART, have no concurrent opportunistic infections and are clinically stable (2, 60, 61, 80, 114, 137, 138, 143, 153, 166, 177, 252). A small subset of these studies examined the effect of IL-2 on patients with moderately advanced disease and CD4+ counts < 200 cells/uL (2, 137, 143, 153, 177). Representative IL-2 treatment regimens are shown in Table 1-1 to demonstrate both the wide range of effective doses and the range of intervals between doses.

In both low and high dose studies, IL-2 administration induced transient spikes in plasma viremia despite HAART that returned to baseline levels soon after administration (60, 137, 143) suggesting, that in the context of HAART controlled

infection, IL-2 has only transient virological effects. However, one report did indicate that in patients with low CD4 T cell counts (< 200cells/mL) HAART+IL-2 therapy can induce a sustained increase in plasma viral load (137). A number of studies have determined that IL-2 administration is associated with increased total CD4+ T cell counts but not increases in total CD8+ T cell numbers (60, 61, 80, 114, 137, 138, 143, 166, 177). The increase in the absolute number of CD4+ T cells has been most easily observed in patients with relatively high initial CD4+ T cell counts (>200 cells/uL), although HAART treated patients with low CD4+ T cell levels have also demonstrated an immunologic benefit (60, 137, 177). To fully assess the immunological impact of IL-2, the effect of the cytokine on individual T cell subsets has been examined. Two populations of CD4+ T cells have been shown to expand following IL-2 therapy, naïve cells (80, 143, 166, 177, 252) and a population of CD4+ CD25+ T cells expressing intermediate CD45RA and CD45RO levels (60, 61, 80, 137, 143, 252). Interestingly, the expansion of the CD4+ cells is polyclonal which suggests that IL-2 may contribute to broadening the T cell repertoire (137). Another benefit of IL-2 therapy is that it has been associated with decreased expression of activation markers such as HLA-DR and CD38 on CD8+ T cells (61, 137, 138, 166). This may be important in HIV infection as aberrant and excessive immune activation has been shown to be a better correlate to disease progression than commonly monitored clinical parameters such as viral load and CD4+ count (78, 146, 155).

The caveat to introducing IL-2 as a widespread immunotherapeutic, despite its consistently promising immunological benefits for patients, is toxicity concerns dating from the earliest studies. Sequelae associated with IL-2 administration range from nausea (61, 137, 138, 143, 177), fever (60, 61, 137, 138), and fatigue (60, 61, 137, 138, 177) to capillary leakage (137), hypotension (138) and myocardial infarction (143). Dose titration studies using IL-2 in patients have been used to determine the maximum safe dose of the cytokine for each patient as that which maintains the increased CD4+ count without inducing toxicity (60, 80, 114, 166). It was hypothesized that the early high dose trials were not only saturating the high affinity IL-2R on T cells, the excess cytokine was binding to and activating an intermediate affinity receptor on NK cells leading to the observed sequelae (114). In support of this hypothesis, two studies in HIV+ HAART treated patients have titrated IL-2 dosing regimens to saturate only the high affinity IL-2R to avoid these negative consequences (114, 166).

With regard to its use as a vaccine adjuvant, endogenous IL-2 has been suggested to increase anti-viral immunity and preservation of IL-2 secretion in activated T cells during SIV has been correlated with slower disease progression (135). These types of observations provide the rationale for using IL-2 as a vaccine adjuvant to increase the efficacy of memory recall responses. IL-2 expressing

plasmids have been used with DNA based vaccines and result in modest increases in SIV specific response (16, 193), preservation of peripheral CD4+ T cells following a homologous SHIV challenge (15, 16), and decreased viral load set point after homologous challenge with a SHIV89.6P virus (15, 16). The true potential of IL-2 as a vaccine adjuvant will likely be determined in human clinical trials, some of which are currently ongoing.

Interleukin-15:

The immunological benefits of IL-15 have been assessed primarily in non-human primate models in which it effectively induced expansion of CD8+ EM T cells (220, 284). Several studies have shown in murine and simian model systems that expression of the IL-15Ra chain on CD8 T cells is not required to generate immunological memory, rather expression of both IL-15 and IL-15Ra from the same antigen presenting cell is required for optimal function of the cytokine (18, 41, 292). These findings suggest a strong role for both trans-presentation and reverse signaling as discussed above, for developing optimal antigen specific recall responses. In a direct comparison of the ability of IL-2 and IL-15 to induce the expansion and retention of antigen specific memory cells in the macaque model, IL-15 was shown to be more effective at inducing new long term CD4 and CD8 memory to flu and tetanus toxins than IL-2, though a combined regimen of IL-2 and IL-15 was superior to either cytokine alone (284). These results suggest

that when properly administered, IL-15 has the potential to benefit an SIV/HIV+ host by both increasing anti-viral immunity and by effectively generating new memory responses for other pathogens. In addition to aiding the development of memory responses in healthy macaques, IL-15 has been clearly shown to induce homeostatic proliferation in both CD4+ and CD8+ EM T cells in the periphery (220) that could be involved in the maintenance of a functional memory response. IL-15 did not induce proliferation in either the naïve or CM T cells in these experiments, however the EM cells generated in the periphery do successfully traffic to mucosa sites (220). This argues that IL-15 may be useful in restoring the CD4+ EM cell population at the mucosa, depletion of which has been implicated both in progression to sAIDS in the SIV+ macaque model and susceptibility to opportunistic infection (220, 284).

The immunologic impact of IL-15 administration has been tested in SIV infected rhesus macaques in which it was successful at inducing expansion of both NK cells and memory T cells. One potential immunological benefit of IL-15 therapy is the regeneration of CCR5+ CD4+ EM T cells, the cells most readily targeted by SIV infection (220). The expansion of this cell population could have two possible outcomes in an ART naïve macaque, the increase in virus targets could lead to increased viral load and exacerbation of the infection, or it could restore the CCR5+ CD4+ EM compartment slowing progression to sAIDS. In ART

naïve SIV+ macaques IL-15 fails to induce proliferation in CD4+ EM cells (220), although proliferation is robustly induced in CD8+ EM cells (188, 189, 220). In the same macaques, ART treatment restores the ability of IL-15 to induce proliferation in CD4+ EM cells (220). The virological effects of IL-15 administration in ART naïve SIV+ macaques appear to be dependent on when the cytokine is administered. During acute infection, IL-15 administration has been shown to increase viral setpoint by nearly three logs, and in 30% of SIV+ macaques increased rate of disease progression, despite increasing the absolute number of both NK cells and SIV-specific CD8+ T cells (188). By contrast, IL-15 administration during chronic infection in ART naïve animals does not appear to increase plasma viral load (189).

The potential for IL-15 to exacerbate disease by increasing viral target cells as well as its preferential impact on CD8+ memory T cells has lead to IL-15 being considered in several studies as a vaccine adjuvant to increase immune responses to SIV/HIV. In this capacity IL-15 has been tested in isolation as well as in combination with other cytokines such as IL-7 and IL-12 (49, 111). Several studies have shown that co-innoculation of an SIV antigen encoding DNA vaccine and an IL-15 coding plasmid increases the number of antigen specific IFN-gamma producing CD8+ T cells (32, 49, 111). Following challenge of the vaccinated animals, the degree of protection from IL-15 coinnoculation was

highly dependent on the virus used for challenge. In a weakly pathogenic SHIV (SIV envelope proteins with an HIV backbone) (SHIV 89.6P) challenge model, the IL-15 adjuvant was associated with lower peak viremia and full suppression of viremia by week 12-post challenge compared to week 26 post challenge for animals given only an SIVgag DNA vaccine (32). Using the same SHIV (89.6P) a second study found that IL-15 coinnoculation resulted in a similar increase in SIVgag specific responses, but clinically the macaques failed to demonstrate protection from CD4+ depletion or a reduction in viral load compared to animals receiving only the SIVgag vaccine post challenge (49). However, when a highly pathogenic virus, SIVmac251, was used as the challenge virus the use of IL-15 as an adjuvant increased viremia compared to the vaccine only recipients and therefore was not effective (111). Overall, the utility of IL-15 for HIV/SIV vaccine or immune-therapy may be very limited, although there may be some potential for it use to improve EM cell levels at mucosal sites of chronically infected HIV+ patients that are effectively suppressed by HAART therapy,

Interleukin-7:

As discussed above, IL-7 has shown a great deal of promise in reconstituting a balanced immune system in non-HIV associated lymphopenia (21, 237, 266). The primary challenges to using IL-7 therapeutically are: 1) Safety; 2) Impact of IL-7 on viral replication; and 3) The efficacy with which IL-7 expands the target

CM T cell population. Indeed, in-vitro analyses demonstrate that IL-7 can increase HIV replication (165, 232, 260, 263). However, multiple studies utilizing the SIV/macaque model have shown that in both treatment naïve and ART treated macaques, IL-7 has no discernable impact on plasma viremia (24, 92, 111, 204). The effect of IL-7 administration on immune activation needs to be further investigated as IL-7 may increase activation markers including Fas, CD69 and HLA-DR expression on peripheral T cells (84). Additionally, in HIV+ humans a positive correlation between high levels of endogenous IL-7 and markers of immune activation/proliferation have also been observed in agreement with the results from the macaque model (84, 172, 192). Proof of principle studies undertake in healthy non-human primates demonstrated IL-7 potently induced proliferation and expansion of the peripheral T cell compartment encompassing both CD4+ and CD8+ T cells of both the naïve and memory subsets (92, 160, 184). The effects of IL-7 in these studies were not limited to the peripheral blood and included both lymph nodes and other organs (eg small intestine, spleen, lung and kidney) (25, 160, 184). IL-7 continues to be efficacious during SIV infection in the macaque model and has been used in both untreated (204) and ART treated (24, 92) SIV+/macaque models. The primary immunological benefit seen in the SIV model from IL-7 therapy has been increases in naïve and CM CD4+ T cells though recent work suggests IL-7 therapy can restore CD4+ T cells at mucosal sites as well (24, 25, 92, 204). The initial NHP studies undertaken using IL-7

were proof of concept studies, that provided the first insights into some of the benefits of IL-7 therapy. However, these initial studies contained some caveats. The first major caveat is that the IL-7 administered to the animals was immunogenic for one of two reasons; it was either recombinant human IL-7 made from bacteria (92, 204) or recombinant macaque IL-7 made from eukaryotic cells that lacked appropriate post-translational glycosylation (24, 65, 184). These studies also administered very high doses of cytokine at high frequency (discussed further in Chapter 6) resulting in saturation the IL-7/IL-7R system leading to downregulation of the IL-7R (24, 92). Finally, the strength of IL-7 as an immunotherapeutic we believe lies in its ability to regenerate a particular subset of CD4+ T cells, the central memory subset, previous studies (92, 204) lack both the sampling and flow cytometric resolution to be able to identify and elucidate the impact of IL-7 administration on the CM subset.

As with IL-2, IL-7 clinical trials are underway assessing both the safety and efficacy of IL-7 treatment in patients. There are currently twelve clinical trials, either ongoing or completed, four are being conducted with HIV patients and the remainder in patients undergoing treatment for cancer. All studies in HIV+ patients thus far have been conducted in patients on stable HAART and transient increases were observed in the absolute number of both CD4+ and CD8+ naïve and memory T cells with no discernable changes in plasma viral loads (149, 251).

Some of these current and future studies will likely utilize IL-7 produced in mammalian cells, allowing for proper glycosylation modifications, which may increase the in vivo half-life and improve bioavailability of the IL-7 protein further increasing its utility as a potential immune therapeutic.

Rationale:

The overarching aim of this dissertation project was to elucidate the interplay between IL-7 function and the maintenance of peripheral T cell homeostasis in the SIV/macaque model. We approach this aim in two ways. First, we sought to determine the efficacy of IL-7 as an immunotherapeutic. The patient population we hypothesize is most likely to benefit from the development of novel treatments is HIV patients exhibiting a discordant response to HAART (discussed above). By proposing IL-7 as an immunotherapeutic in this population we are specifically seeking to increase the number of CD4+ CM cells in the periphery. This dissertation assess the ability of IL-7 to restore CD4+ CM cells, as well as other T cell subsets, in the SIV+ macaque model. Our work is the first systematic attempt to determine the dosing regimen that maximizes expansion of the CM compartment specifically. In addition, we designed our study to avoid the caveats associated with previous investigations. The IL-7 we use in our studies was generously provided by Cytheris Inc and is recombinant macaque IL-7 produced in mammalian CHO cells and is guaranteed to be glycosylated on 3/5 possible N-

linked sites and 1/1 O-linked sites. In house trials by Cytheris, in addition to our work and a study published this year (25) indicate that this IL-7 is non-immunogenic and well tolerated in the macaques. In addition, we designed our protocol to incorporate "wash-out" periods during the IL-7 administration hypothesizing that this would prevent saturation of the IL-7R. For the same reason, to avoid IL-7R downregulation, we also used a comparatively low dose of IL-7 (discussed further in Chapter 6). Further, we expand on the results presented in previous work by assessing the impact of IL-7 administration on alternative immune cells, in this case B cells and double negative T cells, populations that have been underappreciated in previous studies. The data presented here provides evidence that IL-7 can be an effective immune therapy in the SIV+ macaques that are on fully suppressive anti-retroviral treatment. These data also provide insights into the factors that contribute to an effective outcome following IL-7 treatment include identification of the optimum timing between doses. Our second approach to understand the interplay between IL-7 and memory T cell homeostasis was to examine the ability of peripheral T cells to respond to IL-7 during untreated SIV infection. The homeostatic failure of the CD4+ CM compartment is hypothesized to be a major determinant of the rate of disease progression in the macaque modeal (208). Therefore, it is of general interest to the SIV/HIV field, as well as specifically for an immunotherapeutic, to understand how T cells respond in-vivo to one of the primary cytokines involved

in regulating their homeostatic survival and proliferation (145, 236, 286). It is also of interest from a therapeutic standpoint in that the ability of peripheral T cells to respond to IL-7 with actively replicating virus in the system affects whether our target patient population requires suppressive HAART concurrent with IL-7 administration. Using the same IL-7 provided by Cytheris (discussed above) we will assess whether IL-7 responsiveness is altered in untreated SIV infection (discussed in Chapter 5). Further, we will assess both cells tht can be readily SIV infected (CD4+ EM cells) as well as cells that are generally not infected by SIV (CD4+ Naïve and CD8+ CM cells) to determine whether factors influencing IL-7 responsiveness are dependent on the ability of IL-7 to infect a cell population. We hypothesize that the loss of IL-7 function stems from aberrant intracellular signaling with consequences for cell survival. The results from this dissertation are generally in agreement with previous studies in the SIV-macaque model and indicate that IL-7 is a potentially potent and targeted therapeutic able to elicit increased CM T cell levels. Further, the increase in T cell levels is independent of robust T cell proliferation and appears to be mediated by the survival function of IL-7, and the positive influence of IL-7 can be inhibited by an active SIV infection.

Table 1-1: IL-2 dosing strategies in Human Clinical Trials

Study	IL-2 dose	Route of administration	# of cycles	Interval
Mitsuyasu et al 2007	9 mIU/day for 5 days	Intravenous	9	8 weeks
	15 mIU/day for 5 days	Subcutaneous	9	
Lalezari et al 2000	1.2 mIU/m2	Subcutaneous	6 months	daily
Leef et al 1996	0.19 - 0.25 mIU/m2	Subcutaneous	6 months	daily
Farel et al 2004 Sereti et al	15mIU for 5 days	Subcutaneous	162-417	2-91 months*
Kovacs et al 1995 Kovacs et al 1996	18 +/- 6 mIU/day for 5 days	Intravenous	6	bimonthly

* The interval was dependent on immunological end points being met; some patients maintained elevated CD4+ counts longer than others

CHAPTER TWO

MATERIALS AND METHODS

Animals and Viruses:

For these studies purpose-bred male Indian origin rhesus macaques (Macaca mulatta) were used. The animals were housed at the Oregon National Primate Center in accordance with standards of the Center's Animal Care and Use Committee and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Macaques enrolled in the cohorts were between 5-7 years of age and deemed free of Cercopithicine herpesvirus 1, D type simian retrovirus, simian T-lymphotropic virus type 1, and SIV infection. 8 macaques remained SIV uninfected over the course of these studies; the remainder (N=27) were infected intravenously with SIVmac239 using 5ng equivalents of SIV p27 (1.0 x105 infectious centers). 10 of these macaques were used during the acute phase of infection to determine the effect of SIV infection on double negative T cells (RM2-02 (ONPRC number: 23772), RM2-05 (22657), RM2-06 (23788), RM2-07 (23201), RM2-08 (23750), RM2-09 (23686), RM2-10 (23092), RM2-11 (23892), RM 2-12 (23699) and RM2-11 (24090)). During chronic infection (>100 days post infection) 13 macaques were treated with anti-retroviral therapy (ART) consisting of PMPA and FTC for 4-6 weeks allowing both viral load and CD4+ T cell levels to stabilize. Some macaques were used in multiple studies;

however, each macaque was rested for a minimum of 10 weeks between sequential protocols so they were able to return to their pre-IL-7 exposure levels in all the measured parameters. The following macaques were enrolled in the dosing interval study: presented in chapter 3 RM2-01 (23185), RM2-02, RM2-03 (23186), RM2-04 (23208), RM2-05, and RM2-06. These animals were treated daily with PMPA (30mg/kg) and FTC (50mg/kg) for 6 weeks prior to rsIL-7gly. Two rsIL-7gly doses were administered one week apart (Table 2-1). A third rsIL-7gly dose was given to 2 macaques at either 2, 4, or 6 weeks after the last rsIL-7gly dose (Table 2-1). RM2-01, RM2-03, and RM2-04 were then used to test the efficacy of a single rsIL-7gly dose at 6-week dosing intervals (Table 2-1) while still being stably treated with PMPA (30mg/kg) and FTC (50mg/kg). To test the efficacy of clustering rsIL-7gly doses two macaques, RM2-05 and RM2-07, received a total of 9 rsIL-7gly injections. Macaques were given three doses of rsIL-7gly administered at weekly intervals followed by a two week "wash-out" during which no rsIL-7gly was administered the series was repeated three times (Table 2-1). ART treated controls for this series of experiments were: RM2-02, RM2-06, RM2-10 and RM2-11 (Table2-1). Both groups of animals (ART and ART+rsIL-7gly) were given 4 weeks of daily ART (PMPA (30mg/kg) and FTC (50mg/kg)) that was reduced to a maintenance dose one week prior to beginning administration of rsIL-7gly (20 mg/kg PMPA; 20 mg/kg FTC) to prevent toxicity. Two animals failed to suppress viremia after initiating ART (RM2-12 and RM2-

13) and two animals lost control of viremia after ART was reduced (RM2-08 and RM2-09) and were not included in the analysis of rsIL-7gly efficacy. Macaques used in the studies presented in chapter 5 (N=14) were not given any ART treatment and had been SIV+ for 100-500 days at the time of rsIL-7gly administration. All macaques were clinically stable when they were enrolled in the cohort with no detectable opportunistic infections. End stage macaques were selected for the study based on two additional criteria; few if any detectable CD4+ CCR5+ EM T cells and Ki67 levels <20% in the CD4+ CM compartment (proliferation collapse). Macaques that developed disease states that were not clinically manageable were euthanized in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. End-stage AIDS was defined by the presence of AIDS-defining opportunistic infections, wasting syndrome unresponsive to therapy, or non-Hodgkin lymphoma.

Interleukin-7 Treatment of Rhesus Macaques

Animals were treated with recombinant macaque Interleukin-7 (IL-7) produced in mammalian cells (CHO) and glycosylated at a minimum of 2/4 possible N-linked sites and 1/1 possible O-linked sites (Cytheris). The animals were followed longitudinally using flow cytometric analysis up to 112 days following treatment with IL-7 to assess changes in immunological and virological parameters. IL-7

(30ug/kg) was administered subcutaneously in the dosing regimens shown in table 2-1. All SIV+ animals, both ART treated and untreated, were given IL-7 during the chronic phase of infection with cytokine administration beginning from 142-492 days post infection.

BrdU treatment

5-bromo-2'-deoxyuridine (BrdU) labeling was used to label cells in-vivo in macaques receiving rsIL-7gly in three dose clusters. 10g of BrdU (Sigma-Aldrich) was prepared in 1L of sterile HBSS in the following manner; BrdU was dissolved completely in the HBSS (pH 7.2) at room temperature. The solution was filter sterilized (0.2 uM filter) and stored in 80mL aliquots (final concentration 10mg/mL, pH 7.2) at -20C. Upon thawing, the solution was mixed vigorously at room temperature prior to administration to resuspend any precipitate. 60mL of the prepared BrdU solution was administered intravenously while the animals was anesthetized at a rate of 3mL/min each day for four days.

Lymphocyte Isolation

Cells for flow cytometry were obtained from whole blood as previously described (289). In addition, both plasma and peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples. To isolate plasma, the whole blood samples were centrifuged at 1800rpm for 20 minutes. Plasma was stored in either

0.5mL or 1mL aliquots at -80C. PBMCs were isolated following plasma isolation by density gradient. The remaining whole blood was diluted to a 30mL volume using Hank's Balanced Salt Solution (HBSS) (Cellgro) and overlaid onto 12-13mL of Lymphocyte Separation Media (Histopaque). The overlaid samples were centrifuged at 3,000rpm for 25 minutes; no brake was used to stop the centrifuge. The lymphocyte layer was collected and washed twice with complete R10 (RPMI-1640 (HyClone)+ 10% FCS (HyClone)+ 2mM L-glutamine (Sigma-Aldrich)+ 1mM sodium pyruvate (Sigma-Aldrich)+ 100IU/mL penicillin/ streptomycin (Sigma-Aldrich)) (2,000 rpm for 20 minutes; 1800rpm for 8 minutes). The cells were frozen in BGS+10% DMSO had stored at -140C or used in additional assays.

Plasma Viral Load Quantification

Plasma viral load was determined using a real time RT-PCR assay as described previously (51). The threshold sensitivity of the assay was 30 SIVgag RNA copy eq/mL.

Flow Cytometric Analysis of T-cell Phenotype

Polychromatic flow cytometry was performed on an LSR-II Becton Dickinson instrument using Pacific Blue, AmCyan, FITC, PE, PETexas, Red, PE-Cy7,

PerCP-Cy5.5, APC, APC-Cy7, and Alexa 700 as the available fluorescent parameters. FlowJo (Treestar) software was used for analysis.

The combinations of markers used to identify each cell population are shown in Table 2-2. From whole blood, naïve and memory T-cell phenotypes were defined using the markers CD28, CD95, CCR5 and CCR7 on gated CD3+CD4+ or CD3+CD8+ T cells. The parameters used to identify naïve and memory T cells have been described in depth previously (221). An example of the gating strategy used to identify sub-populations in both CD4+ and CD8+ T cells is shown in figure 2-1 and figure 2-2 respectively. Briefly, naïve T cells are a uniform population expressing the following combination of markers; $CD28^{moderate}$, $CD95^{low}$, $CCR7^{moderate}$, $CCR5^{-}$, in both CD4+ and CD8+ T cells. The memory T-cell phenotype is more diverse but is generally $CD95^{high}$ and displays one or more non-naïve cell phenotypes, $CD28^{-}$, $CCR7^{-}$ or $CCR5^{+}$. CM and EM are addressed most prominently in these studies are defined in the following manner in both CD4+ and CD8+ T cells; CM: $CD95^{high}$, $CD28^{high}$, $CCR5^{-}$, $CCR7^{+}$ and EM: $CD95^{high}$, $CD28^{-}$, $CCR5^{+}$, $CCR7^{-}$. Double negative T cells were identified as (CD3+CD4-CD8-pan$\gamma\delta^{-}$).

Detection of BrdU labeled Lymphocytes

BrdU labeled lymphocytes from the blood were detected by flow cytometry as described previously (221). $1e^6$ lymphocytes were stained per test. Briefly, lymphocytes were stained for surface phenotype markers for 1 hour at room temperature. The cells were then fixed for 10 minutes at room temperature using FACSlyse solution (1mL, BD Biosciences). Cells were then washed once in 1x PAB and permeabilized twice (0.5mL Fix Perm solution (BD Biosciences), 10 minutes at room temperature, wash with 1x PAB). Following the permeabilization step, cells were incubated with 0.28mg of DNAse-1 (Sigma-Aldrich) and a directly conjugated BrdU specific monoclonal antibody for 30 minutes at room temperature. The specificity and criteria for determining positively stained cells was determined by comparison to BrdU staining from the same animals prior to BrdU labeling.

Detection of Phosphorylated STAT5 by Flow Cytometry

Phosflow, flow cytometric detection of phosphorylated residues on specific proteins, was performed on PBMCs ex-vivo using a protocol developed with Becton Dickinson. $2e^6$ PBMCs were washed once with 5mL phosphate buffered saline (PBS) and centrifuged at 2,000rpm for 10 minutes in a 15mL conical. The surface phenotype cocktail (CD3, CD4, CD8a, CD28, CD95 and CCR7) was added to the cells for 30 minutes at room temperature. Following the surface stain, PBMCs were stimulated with recombinant rhesus macaque IL-7 (Cytheris)

in a dose curve ranging from 0-16ng/1e^6 cells for 15 minutes at 37C in a 1mL volume of PBS. The cells were fixed following IL-7 by adding 140uL of 40% paraformaldehyde (Sigma) while vortexing. The cells were incubated in PFA for 10 minutes at 37C and then washed with 10mL PBS. Following resuspension of the pellet 3mL of 1x BD custom phosflow permeabilization buffer was added and the cells incubated at room temperature for 30 minutes. The cells were washed twice in PBS+1%BFA and stained intracellularly with Ki67 (FITC) and STAT5-pY603 (PE) for 1 hour at room temperature. After washing once with PBS+1%BFA, the cells were transferred to 5mL polystyrene flow tubes and collected on an LSR II. The specificity and criteria for determining positively stained cells was determined by comparison to an unstimulated sample for each animal.

mAbs

The following fluorophore conjugated monoclonal antibodies were obtained from Beckton Dickinson Biosciences: SP34-2 (CD3; Alexa 700), L200 (CD4; AmCyan), SK-1 (CD8a; PeCy-7, PerCP-Cy5.5, APC-Cy7), DX2 (CD95; PE, PE-Cy7), hIL-7R-M21 (CD127; PE), 3A9 (CCR5; APC); B56 (Ki67; FITC, PE); L27 (CD20; True Red); B44 (BrDU; FITC) STAT5P (PE). The clone 28.2 (CD28; PE-TexasRed) was obtained from Beckman Coulter. The purified, unconjugated antibody 15053 (CCR7) was obtained from R&D Systems and conjugated in

house to biotin using the Pierce Biotinylation kit. CCR7-biotin was detected using a pacific blue conjugated streptavidin from Invitrogen. FN-18 (CD3) was produced and purified in house and conjugated to Alexa 700 using an Invitrogen conjugation kit.

Determination of Plasma Concentration of Cytokines using Luminex

Multiplex kits for measuring the plasma concentrations of IL-2, IL-7, IL-15 and IL-8 were obtained from Millipore. The kits were used per manufacturers instructions. Frozen plasma samples from SIV+ macaques were rapidly thawed and clarified by centrifugation at 10,000xg for 5 minutes. 25uL of plasma were used per well in a 96 well filter plate. Incubations were performed with agitation on a plate shaker overnight at 4C. After the final wash, beads in the 96-well microtiter plate were resuspended in 125uL of sheath fluid and loaded onto the Luminex instrument. The acquisition gate was set between 8,000-15,000 to discriminate single and doublet events and a minimum of 100 events was recorded for each region. Cytokine concentrations were determined using a standard curve generated with recombinant cytokines and a weighted 5-parameter logistic method.

SIV envelope-specific antibody endpoint titer

Antibody reactivity to detergent disrupted SIVsmB7 envelope proteins were determined in a conconavalin A (ConA) ELISA as previously described (52). Briefly, SIVsmB7 viral envelope proteins (gp120 and gp41) were captured onto 96 well microtiter plates (Immulon 2HB; Dynex Technologies, Chantilly, VA) coated with 5 µg of ConA/well for 1 h at 25°C. After a washing step with phosphate-buffered saline (PBS), non-specific binding was blocked by the addition of 5% dry milk in PBS (blocking solution) to all wells and incubated for 1 h at 25°C. Heat-inactivated plasma samples were serially diluted in blocking solution and incubated in the SIVsmB7 envelope-coated wells for 1 h at 25°C. After an extensive washing, peroxidase-conjugated anti-monkey IgG (Nordic Immunology Laboratories, Tilburg, Netherlands) was diluted in blocking solution, added to each well, and incubated for 1 h at 25°C and washed. Following the final wash step, all wells were incubated with TM Blue substrate (Seracare, Milford, Mass.) for 20 min at room temperature, color was developed by the addition of 1N sulfuric acid, and colorimetric analysis of antibody binding to SIVsmB7 was performed at an optical density of 450 nm (OD450) using a Spectra Max 340 PC (Molecular Devices, Sunnyvale, CA).

Statistical Analyses

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). To calculate change in Ki67 and absolute T-cell

number baseline is shown as the average of three pre-IL-7 timepoints. The two tailed Mann-Whitney t-test was used to calculate the differences between responses to rsIL-7gly. Statistical significance was attained at $p<0.05$ (95% confidence interval). Exact p values for significant differences are shown.

TABLE 2-1- Rhesus Macaque Cohorts and IL-7 Dosing Regimens

Study Title	# of RMs	ID#	Total doses	Interval between doses	Days rsIL-7gly given	Thesis Chapter
IL-7 treatment in SIVneg macaques	8		2	2 doses of IL-7 at a one week interval	0,7	3,5
SIV+ IL-7 treated NO ART	14		2	2 doses of IL-7 at a one week interval	0,7	5
SIV+ ART treated controls	4	RM2-02 RM2-06 RM2-10 RM2-11	0	n/a	n/a	3,4
rsIL-7gly at 2, 4 or 6 week intervals	6	RM2-01 RM2-02 RM2-03 RM2-04 RM2-05 RM2-06	3	2 doses at one week interval (N=6); followed by one dose at 2, 4 or 6 week intervals (N=2)	0,7 21or 35 or 49	3
rsIL-7gly 6-week intervals	3	RM2-01 RM2-03 RM2-04	3	One IL-7 dose every 6 weeks	0,42,84	3
clustered rsIL-7gly doses	4	RM2-05 RM2-07	9	3 doses at one week interval followed by a two week "wash-out"; repeated three times	0,7,14, 35,42,49, 70,77,84	3,4

TABLE 2-2- Antibody Staining Panels used for Phenotypic and Phos-flow Flow Cytometry

	Pacific Blue	Am-Cyan	FITC	PE	Tx Red	True Red	PE-Cy7	APC	Alexa 700	APC-Cy7
Naïve and Memory T-cell phenotype	CCR7	CD4	Ki67	CD95	CD28	CD8a		CCR5	CD3	
CD127 Expression	CCR7	CD4	Ki67	CD127	CD28	CD8a		CD25	CD3	
Detection of BrdU labeled cells		CD4	BrDU	Ki67	CD28	CD20	CD95	CCR5	CD3	CD8a
Effect of IL-7 on DN T cells	CCR7	CD4	pangd	Ki67	CD28	CD20	CD8a	CD95	CD3	
Effect of IL-7 on B-cell subsets	IgD		Ki67	CD27	CD19	CD20		CD95	CD3	
Detection of STAT5P	CCR7	CD4	Ki67	STAT5-P	CD28		CD8a	CD95	CD3	

FIGURE 2-1: CD4+ Naïve and Memory T-cell gating strategy

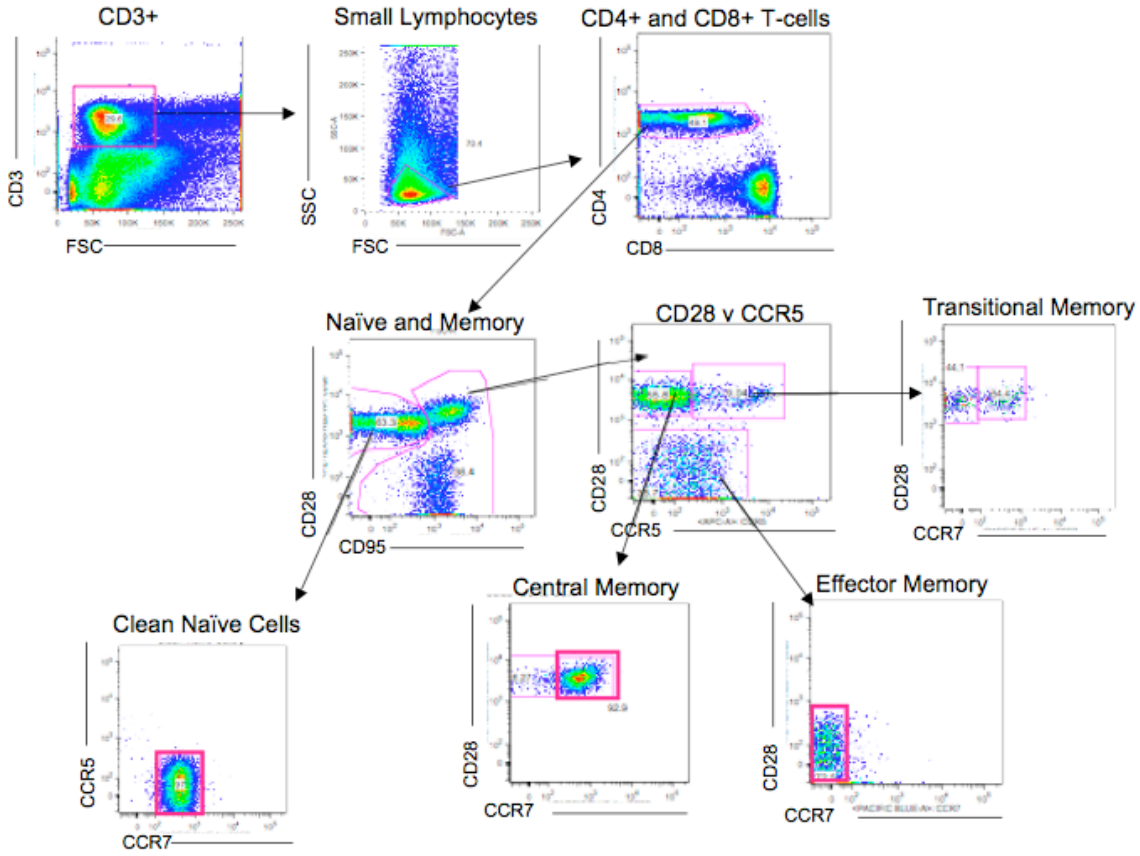
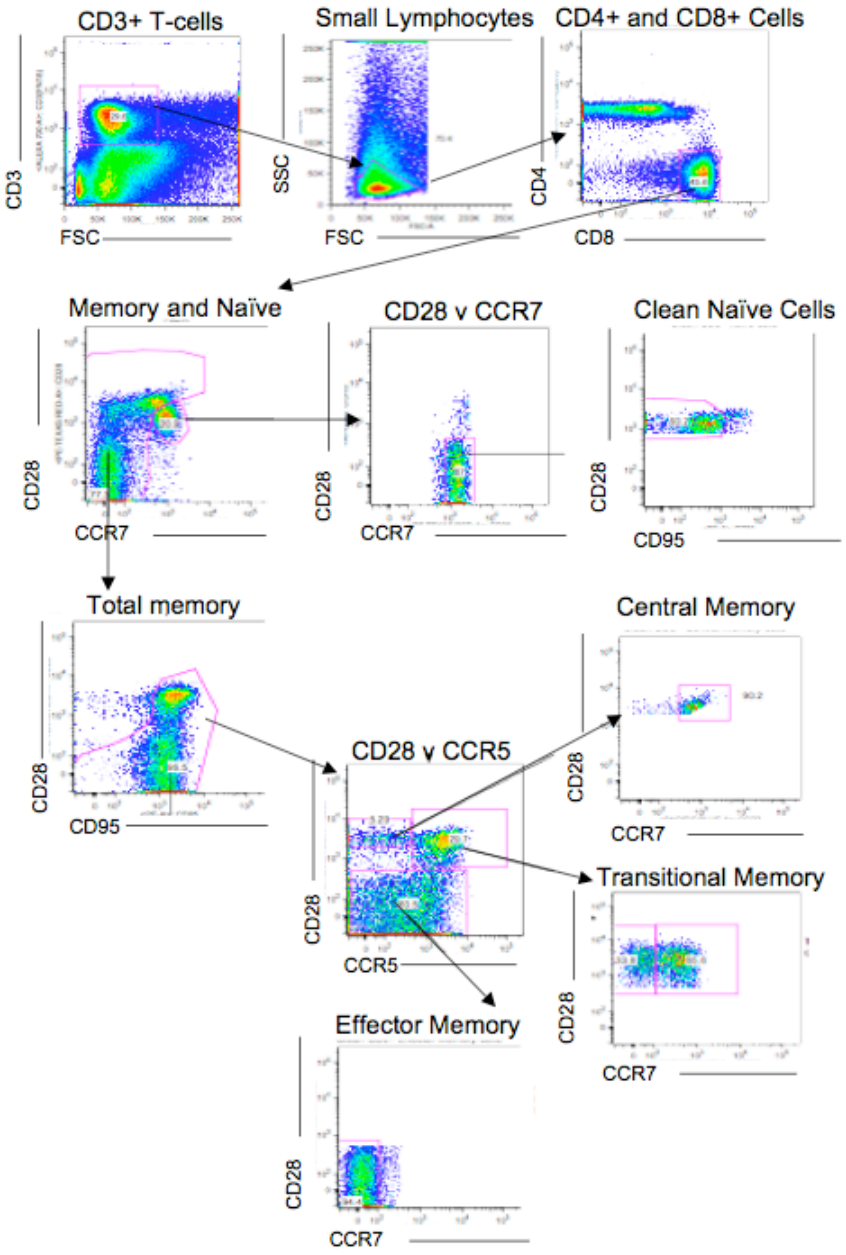


FIGURE 2-2: Gating strategy to Identify Naïve and Memory CD8+ T-cell Subsets



CHAPTER THREE

INCREASED CD4+ T CELL LEVELS DURING IL-7 ADMINISTRATION OF ART TREATED SIV+ MACAQUES ARE NOT DEPENDENT ON STRONG PROLIFERATIVE RESPONSES

Introduction and Rationale:

The primary aim of the work presented in this thesis is to assess the efficacy of rsIL-7gly as a therapeutic. While the best case scenario is the full suppression of viral replication and increased numbers of CD4+ T cells 5-20% of HIV patients exhibit a discordant response to initiation of highly active anti-retroviral therapy (HAART) resulting in an effective suppression of viral replication but only minimal CD4+ T-cell recovery (4, 113, 167). Additionally, in some patients, despite complete viral suppression, T cell reconstitution occurs over years leaving patients susceptible to potentially fatal opportunistic infections (288). Following the initiation of HAART T cell reconstitution in immunological responders (CD4+ T cells increase) is highly dependent on the baseline CD4+ T cell levels at their nadir point, the level of residual immune activation and thymic output (144, 288). In long-term clinical studies of immunologically responsive adults with high CD4+ counts (>200cells/uL) prior to HAART 'normal' CD4+ T cell levels were attained within one year of beginning suppressive HAART (288), by contrast, adults with low CD4+ counts (<200 cells/uL) at the initiation of therapy took up to 7 years to attain 'normal' levels despite complete viral suppression

(288). If successful, IL-7 therapy could benefit both discordant patients and patients with slow reconstitution by increasing CD4+ T cell levels above the levels attainable with HAART alone.

While IL-7 therapy would not be able to alter the nadir T cell levels the primary rationale for using IL-7 as an immune therapeutic in discordant patients is its non-redundant role in production and maintenance of naïve and CM CD4+ and CD8+ T cells (34, 92, 145, 191, 196). The role of naive cells in recovering the CD4 compartment is clearly shown in long-term studies comparing the time to T cell reconstitution between adults and children (288). Pediatric patients recover 'normal' T cell levels within one year of beginning suppressive HAART regardless of the nadir CD4+ T cell levels (288). The reconstitution in pediatric patients reflects increased production of naïve cells above 'normal' levels while memory cells are significantly less than expected in age-matched controls (288). By contrast, adult HIV patients as discussed above, show a wide range of time (1-7 years) to CD4+ T cell recovery and the cell populations that recover in these patients are predominately memory cells (288). Further, discordant patients harbor a disproportionate number of CXCR4 tropic HIV strains that selectively kill mature naïve CD4+ T cells in the periphery (63, 64). IL-7 may be able to restore a more balanced T cell pool in pediatric and adult HIV patients as reconstitution occurs by increasing both naïve and memory cells simultaneously.

Immune activation levels are the third factor that influences the recovery of the CD4+ T cells (63, 144, 288). The importance of immune activation in impairing the T cell compartment both in terms of level and function can be observed in patients that exhibit high viremia and CD4+ T cell recovery following the initiation of HAART (144, 217). In the absence of fully suppressing viremia HAART restores memory T cell responses to specific antigen (58, 217) and protease inhibitors in particular can reduce levels of apoptosis in CD4+ T cells (144), both authors propose indirect reduction of immune activation as one possible mechanism. Discordant patients have also been reported as having elevated levels of IFNgamma, IL-5 and sCD30, markers of activation, than patients that do increase CD4+ T cell levels following HAART (144). In HIV patients we propose that IL-7 may be able to function as a dual purpose immune therapeutic assisting the recovery of CD4+ T cells by increasing both the generation of naïve cells and further expanding depleted memory CD4+ T cells to potentially aid in reconstituting effector sites repairing damage resulting directly from the viral infection.

IL-7 administration to both cancer and HIV+ patients has demonstrated increases in CD4+ T cells indicating that IL-7 therapy can increase our target naïve, and CM CD4+ T cells (149, 237, 251, 266). The study presented here was designed to further optimize IL-7 as a candidate immunotherapeutic with the following

aims: 1) Increasing CD4+ T cells without inducing increased SIV viremia; and 2) Determining the most beneficial strategy to elicit sustained increase in T cell levels, strategy in the SIV-macaque model. These data also provide insights into the factors that contribute to an effective outcome following IL-7 treatment include identification of the optimum timing between doses.

Results:

rsIL-7gly administration in SIV-negative Rhesus macaques induces T-cell proliferation and increases T-cell numbers in the circulation.

Previous work has demonstrated that both non-glycosylated and glycosylated recombinant macaque interleukin-7 (IL-7) exhibited some efficacy at inducing proliferation and expansion of peripheral blood T cells (24, 25, 92, 184, 204). Here the assessment of glycosylated Rhesus macaque IL-7 (rsIL-7gly) was initially undertaken in a two dose regimen (30ug/kg/d) developed in conunction with Cytheris Inc. to assess proliferation in-vivo in peripheral CD4+ and CD8+ T cells in uninfected macaques. Flow cytometric evaluation of the percentage of cells expressing the nuclear antigen Ki67 (a marker for cycling through S phase within the previous 4-7 days (221) revealed a consistent and robust rsIL-7gly associated increase in the percentage of Ki67+ CD4+ naïve (N) and central memory (CM) cells in the blood. This increase was first observed 2-3 days after the initial rsIL-7gly dose (Figure 3-1A) and in naïve cells peaked following the second rsIL-7gly at d12-14. CM cells were more rapidly responsive, exhibiting two peaks, each 5-7 days post rsIL-7gly administration (Figure 3-1A). For both subsets, the percentage of proliferating cells returned to baseline by day 21 (Figure 3-1A). It is noteworthy that whereas the responsiveness to rsIL-7gly was similar between CD4+ and CD8+ CM, among naïve cells, CD8+ cells were considerably more responsive than their CD4+ counterparts. rsIL-7gly administration was also associated with a transient increase in the proliferation of

CD4+ and CD8+ TrM and EM, although for the EM subset, this responsiveness was typically weaker and more variable (Figure 3-1A). As EM generally exhibit low levels of IL-7 receptor (CD127) on their cell surface (10, 22, 53, 131, 172, 183, 184, 212, 233, 242, 281, 301), this proliferation likely reflects subsequent differentiation of rsIL-7gly responding CM and TrM, rather than a direct effect on pre-existing EM themselves. Importantly, irrespective of the T cells subset assessed, no significant difference was observed in Ki67 expression between the uninfected and SIV+ ART treated macaque indicating that in macaques with successful ART rsIL-7gly is able to induce proliferation in our target cell populations, CD4+ naïve and CM cells (grey line compared to black line, Figure 3-1).

We next assessed the ability of rsIL-7gly administration to influence the number of peripheral blood T cell subsets in the uninfected and ART treated SIV+ macaques. As shown in Figure 3-2, the fold change in the CD4+ CM peripheral T cells was comparable in the uninfected and ART treated SIV+ macaques, however for the other CD4+ T cell subsets the levels of T cells were higher in the uninfected macaques (Figure 3-2A). The CD8+ T cells exhibited evidence for an increased response in the uninfected macaques compared SIV+ ART treated macaques for all four of the cell subsets assessed (Figure 3-2B). Overall the elevation in CD4+ and CD8+ T cell levels was quite transient in the naïve and

EM T cell subsets, but were retained to some degree throughout the 21-day follow-up in the CM and TrM T cell subsets (Figure 3-2A-B). In the following experiments, while we recognize the importance of naïve cells to CD4+ reconstitution, the major focus will be to assess the CM compartment as these cells are the most responsive to rsIL-7gly (ΔKi67 35% vs ΔKi67 12% in naïve cells; 1.9 fold increase at day 28 vs 1.5 fold increase in naïve cells) in SIV+ ART treated macaques.

Timing of rsIL-7gly administration in ART treated SIV+ Rhesus macaques – Dosing Interval Assessment and IL-7 treatment at 6-week intervals

We sought to determine the optimal spacing between sequential rounds of rsIL-7gly treatment so as to maximize expansion of CD4+ CM, the core subset of the depleted memory T-cell compartment of SIV-infected macaques. The absolute number of T cells in each macaque after reaching stable levels on ART and prior to rsIL-7gly is presented to enable a more comprehensive understanding of the data regarding the change in absolute CD4 T cell numbers (Table 3-1). Six SIV-infected macaques were treated first with two doses of rsIL-7gly seven days apart, and then the T-cell proliferative response to a third dose of IL-7 after either 2, 4 or 6 weeks was determined (Table 2-1, rsIL-7gly 2,4,or 6 week intervals). Plasma SIV levels were not materially affected by the rsIL-7gly administration in these animals, although transient minor increases in plasma viral loads were observed at day 7 in three of six macaques (increasing from approximately $1x10^{\wedge 2}$ to $1x10^{\wedge 3}$)

(Table 3-2). All six macaques exhibited robust proliferation in the CD4+ CM cells in response to rsIL-7gly on day 0 and day 7 as expected (Figure 3-3A-C). Administering rsIL-7gly six weeks after the previous exposure elicited robust proliferation in CD4+ and CD8+ CM subsets following the third rsIL-7gly dose in RM2-05 and RM2-06 (Figure 3-3A). In both macaques following the initial two doses (day 0 and day 7) there was a transient increase in CD4+ CM T cell levels indicating some benefit from the rsIL-7gly (Figure 3-3D). In addition, the CM T cell levels increased after the third dose 6 weeks later (Figure 3-3D). However, they were transient in one of the macaques (RM2-06) although the second macaque (RM2-05) retained elevated levels 112 days post treatment (Figure 3-3D). These data suggest that a 4-6 week interval between rsIL-7gly doses would be optimal to consistently maximize proliferation, though not necessarily levels, of CD4+ CM T cells. Indeed, rsIL-7gly treatment at this interval also elicits robust proliferation in CD8+ naïve and CM cells as well (Figure 3-3A) indicating that rsIL-7gly is fully functional in all target populations. Administration of the third rsIL-7gly dose at more closely spaced intervals, either 2 or 4 weeks, was associated with varied proliferative responses ranging from robust (RM2-01 DKi67= 20.0%; RM 2-03 DKi67= 15.3%) to attenuated (RM2-02 DKi67= 2.3%; RM2-04 DKi67= 7.8%) in the CD4+ CM subset. One macaque from the 4 week interval (RM2-04) (Figure 3-3B) and one macaque from the 2 week interval (RM2-02) (Figure 3-3C) showed an attenuated proliferative response suggesting

that administering rsIL-7gly at either of these intervals is not optimal to maximize proliferation in the CD4+ CM compartment. This finding is corroborated by similar trends in the CD8+ CM and naïve subsets, which are also less responsive to rsIL-7gly at these intervals (Figure 3-3B and 3-3C) than when rsIL-7gly is given at 6-week intervals (Figure 3-3A). Once again transient increases in the absolute levels of peripheral CD4+ CM T cells could be observed prior to day 21 in all four animals (Figure 3-3E and 3-3F). Following the third dose levels of CD4+ CM cells remained elevated beyond the end of the 112-day study period in one macaque from each group (RM2-03; Figure 3-3E and RM2-01; Figure 3-3F). Interestingly, RM2-01, a macaque that received rsIL-7gly on days 0, 7, and 21 displayed the highest increase in T cell levels at day 112 (RM2-01= 1.77 fold increase CM cells) when compared to the other five macaques (range from 0.55-1.64 fold increase). We hypothesized that if consistently eliciting robust rsIL-7gly induced proliferation after each dose is necessary for optimal expansion of T-cell levels over time with multiple doses then providing rsIL-7gly at 6-week intervals, as we did with RM2-05 and RM2-06, would be predicted to result in the greatest long-term T-cell increases in peripheral blood. By contrast, RM2-01 was given closely spaced rsIL-7gly doses and had the highest increase in T cell levels, leading to an alternative hypothesis, that closely spacing, or clustering doses, may be beneficial even if the proliferative response to each dose is more varied.

To test whether the 6-week interval administered to RM2-05 and RM2-06 would elicit long-term benefits after repeated exposures, we administered one dose of rsIL-7gly to three ART treated SIV+ macaques at 6 week intervals (Table 2-I, rsIL-7gly 6-week intervals, 3 total doses). The absolute number of T cells in each macaque after reaching stable levels on ART and prior to rsIL-7gly is shown in Table 3-1. This dosing regimen was not associated with any increase in the SIV plasma viremia in these SIV+ ART treated macaques (Table 3-2). Peak Ki67 expression occurred 5-7 days post injection and returned fully to baseline by day 14 (Figure 3-4). As predicted from the first dosing study (Figure 3-3), the second and third IL-7 administrations given at 6-week intervals resulted in increased Ki67 expression that was generally comparable to the initial IL-7 response in the CD4+ CM subset (Figure 3-4A). The absolute number of CD4+ CM T cells increased as well, averaging 2-2.5 fold increases from baseline levels, in the peripheral blood synchronous with increased Ki67 expression (Figure 3-4C). rsIL-7gly administration resulted in only a small increase in Ki67 expression in the CD4+ naïve cell subset (Figure 3-3A). The absolute number of CD4+ naive T cells did however increase in the blood following each dose (Figure 3-4C). In peripheral blood CD8+ T cells Ki67 expression was elevated in both naïve and CM cells at time points just following the rsIL-7gly administration (Figure 3-4B). In both subsets the increase in the absolute number of T cells was generally transient and returns to basal levels by 21 to 42 days post rsIL-7gly administration

(Figure 3-4C and 3-4D). These data indicate that administration of rsIL-7gly in six week dosing intervals results in induced proliferation of the CD4+ CM subset, however this therapy failed to elicit long-term increases in CD4+ T cell levels.

Assessment of rsIL-7gly utilizing a three dose cluster strategy in ART treated SIV+ macaques

Our final dosing strategy administered 9 total doses over 12 weeks (84 days) to two macaques with ART controlled SIV infection. Two macaques on stably suppressive ART were given three clusters of closely spaced rsIL-7gly doses mimicking the treatment received by RM2-01 in the dosing interval study. Each cluster consisted of 3 doses one week apart with a three-week "wash-out" period between each cluster. The absolute number of T cells in each macaque after reaching stable levels on ART and prior to rsIL-7gly is shown in Table 3-1. As in the previous two dosing strategies, treatment with rsIL-7gly was not directly associated with any increases in plasma viral levels (Table 3-2). The initial three dose cluster of rsIL-7gly administration elicited increased Ki67 expression in CM CD4+ (Figure 3-5A) and CD8 + T cells (Figure 3-5B). The increase in the percentage of Ki67+ cells resulted in a corresponding increase in the absolute number of proliferating CM cells (CD4+: Figure 3-5C and CD8+: Figure 3-5D). Peak Ki67 expression was attained approximately 7 days post rsIL-7gly administration and remained elevated until day 28 suggesting that clustered dosing elicited proliferation over a longer time than a single dose of rsIL-7gly

(Figure 3-5A and 3-5B). Unlike the previous study (Figure 3-4), rsIL-7gly induced proliferation in both CD4+ (Figure 3-5A) and CD8+ (Figure 3-5B) naive T cells, though Ki67 expression was higher in the CD8+ N cells. Interestingly, the rsIL-7gly induced Ki67 increase in CD4+ and CD8+ subsets was attenuated in the second and third dose clusters (Figure 3-5A-D). This attenuated rsIL-7gly response could be observed in each of the T cell subsets with the naïve cells being most affected. The observed reduction in the Ki67 response appeared to be unrelated to CD127 (IL-7R) expression as the percentage of CD127+ cells was similar at the initiation of each cluster (data not shown). Therefore, the clustered dose strategy for administering rsIL-7gly resulted in a robust increase in the number of proliferating cells during the first three dosages that was strongly reduced during the next six rsIL-7gly administrations.

The ability of rsIL-7gly administered in the clustered doses to impact peripheral blood T cell levels was assessed within the different T cell subsets. The clustered dosing regimen successfully increased peripheral blood T cell levels in both CD4+ and CD8+ T cells within each of the virally suppressed SIV+ macaques in the naïve and CM subsets to levels seen in healthy macaques (Figure 3-6A and 3-6B; black lines). Comparing the increase in T-cell number to macaques receiving only ART demonstrates that the effect is rsIL-7gly mediated (Figure 3-6A and B, black lines compared to grey line). This dosing regimen successfully increased

the T-cell levels in the blood compared to ART alone, particularly the CD4+ CM cells, for the entirety of the 112-day study (Figure 3-6A). In addition, despite low levels of induced Ki67 expression (Figure 3-5A) naïve CD4+ T cells also increased in the circulation (Figure 3-6A). Likewise, CD8+ CM and naïve cells showed sustained long-term increases in absolute T-cell number (Figure 3-6B). In summary, these data indicate that clustering doses of rsIL-7gly in SIV+ ART treated macaques induced proliferation in a broad range of peripheral T-cell subsets. Furthermore, the ability to induce a sustained rsIL-7gly related increase in T cell levels appears to be independent of the optimal induction of a Ki67 response in peripheral T-cell.

Assessment of BrDU retention in SIV+ ART and rsIL-7gly treated in weekly dosing clusters in peripheral blood.

To determine whether the rsIL-7gly administrations impacted the retention of proliferating cells we utilized the molecule bromo-deoxy uridine (BrDU) that is incorporated into the DNA of dividing cells and detected by flow cytometry. BrDU was administered for four days following the first of the nine rsIL-7gly doses in the clustered rsIL-7gly dosing study (Table 2-1), as a means of labeling dividing cells. We assessed the rate at which the label was lost in memory T cells in the blood following rsIL-7gly dosing. To determine the rate of loss, the amount of BrDU detected in the T cells was normalized between macaques by setting the percentage of BrDU+ cells at one day post labeling at 100%. In the

CD4+ memory cells, the BrDU label declined to 50% of the original amount by 14 to 28 days post-labeling in SIV+ macaques treated with ART only (depicted in grey) but reached 50% at 42 and 63 days post-labeling in macaques treated with rsIL-7gly (depicted in black) (Figure 3-7A). rsIL-7gly administration had a greater impact on memory CD8+ T cells which retained the label past the end of the follow up period in macaques treated with rsIL-7gly but not in those treated with ART alone (Figure 3-7B). Therefore, in this relatively small number of SIV+ ART treated macaques, the addition of rsIL-7gly to these macaques increased the retention of the CD4+ and CD8+ memory cells that had been labeled during the first clustered weekly dosing.

Discussion:

Current HAART regimens reduce the viral burden but in some patients can be insufficient to restore CD4+ T cells to healthy levels (4, 167). These discordant patients are most likely to benefit from therapy options designed to compliment the existing HAART regimens to restore the host's system to a more "normal" state. IL-7 has been proposed as one candidate to reconstitute the immune system, by increasing CD4+ T-cell levels, complimenting anti-viral drugs during HAART (7, 92, 148, 206). Importantly for the use of IL-7 as a therapeutic, previous studies in the macaque model, as well as our work, have shown that in both treatment naïve and ART treated macaques, IL-7 has little discernable

impact on plasma viremia (24, 92, 111, 204). These studies have also contributed a great deal to documenting the expansion of pathogenically and therapeutically relevant cell populations including the CD4+ naïve and CM subsets (24, 92, 111, 160, 184, 204). In non-human primate models, the effect of IL-7 occurred in both the CD4+ and CD8+ T cell compartments (24, 65, 92, 111, 160, 184, 204). The effects of IL-7 in these studies were not limited to the peripheral blood and included both lymph nodes and other organs (eg spleen, lung, kidney and gut) (25, 160, 184). Most of these studies however used nonglycosylated rmIL-7 (24, 65, 92, 111, 160, 184, 204) and in some instances, the animals developed neutralizing antibodies (24, 65, 204). In our studies with rsIL-7gly all the macaques were monitored for neutralizing antibody production and none was detected. More recently, clinical studies in humans have also been undertaken, one in cancer patients by Sportes et al (266) and two in HIV+ patients conducted by Levy et al (149) and Sereti et al (251). Both Sportes et al and Levy et al document significant though transient expansion of CD4+ and CD8+ T cells lasting from 28 days (266) to 48 weeks (149). Notably, in agreement with the non-human primate model (24, 65, 92, 111, 160, 184, 204), the human studies indicate IL-7 administration effectively expands both naïve and memory T cells (149, 266). Although both human studies reported increased cell cycling, the study by Sportes et al also indicated a role for IL-7 mediated T cell survival effects, detected as

elevated levels of the anti-apoptotic protein BCL-2, as opposed to proliferative effects, as the principle contributor to CD4+ T cell expansion.

Our approach in these studies was to use the SIV/macaque model to further elucidate the efficacy of immune therapy using low doses of rsIL-7gly in a context where ongoing virally mediated killing was minimized. We sought to characterize further in the animal model rsIL-7gly's impact on different T cell subsets using different dosing intervals. Based on previous studies indicating that IL-7 induced both cycling (12, 25, 126, 152, 272) and cell survival (25, 117, 228, 235, 249), to increase cell number, we first conducted a study to determine the optimal spacing between doses. Our results indicated that to consistently maximize rsIL-7gly induced proliferation doses should be administered at 4 to 6-week intervals, however both the duration of the initial proliferative response and the degree to which cell numbers increased was greater when rsIL-7gly doses were administered in clusters. To test these hypotheses we conducted trials of two dosing strategies, one to maximize proliferation, administering rsIL-7gly every six weeks and the other clustering rsIL-7gly at weekly intervals. We repeated each dosing regimen for three cycles assessing the effect of rsIL-7gly on CD4+ and CD8+ naive and CM cells after each dose. When we administered rsIL-7gly at 6-week intervals, we elicited robust proliferation, but little long-term increase in T-cell number. This observation suggests that the cumulative effect of

clustering rsIL-7gly doses may be important to maintaining the self-renewing capacity of CM cells. Two further observations from these studies support the hypothesis that mechanisms other than inducing detectable increases in proliferation may contribute to increasing absolute cell number in macaques. First, CD4+ naïve cells increased absolute count in the periphery with little detectable change in Ki67 expression. Second, when IL-7 was administered in a three-dose cluster, despite observing a muted Ki67 response to the second and third rounds of IL-7 treatment, in the same macaques, the absolute T-cell count continued to increase with each sequential cluster of IL-7 administration. These results suggest that IL-7 has therapeutic benefits contributing to expanding the peripheral T-cell pool that are not the direct result of detectable proliferation.

The importance of maintaining the self renewing capacity of the CM CD4+ T cell population with regard to disease progression has been described in previous studies (208, 225, 285). These data suggest IL-7 administration can effectively regenerate the CD4+ CM subset directly countering a major mode of viral pathogenesis. Further, the results from our BrDU labeling studies indicate that the cells dividing during the first round of rsIL-7gly have a longer retention time in the peripheral blood. This indicates a role for rsIL-7gly in preventing apoptosis and prolonging the lifespan of memory CD4+ T cells suggesting that rsIL-7gly may be able to slow the rate of depletion and consequently disease progression in

the SIV/macaque model. A recent study by Beq et al has further demonstrated that by inducing trafficking to mucosal sites, like the intestine, rsIL-7gly may actually be able to repair SIV induced damage at these sites as well (25). IL-7 administration impacts naïve T-cell population levels in addition to memory cell populations. Increasing the absolute number of naïve T cells allows IL-7 to fully compliment existing HAART regimens in patients that are associated with fewer naïve cells relative to memory cells in adults (288). It could also potentially provide ancillary immunological benefits, such as increased resistance to newly encountered pathogens or increased memory precursor populations that may be beneficial for patients.

In summary, with the work presented in this thesis we have demonstrated that SIV+ macaques with an ART controlled infection respond to rsIL-7gly in a manner similar to what has been observed in SIV-negative macaques with transient proliferative bursts and a potential for a sustained increase in multiple CD4+ T cell subsets at multiple tissue sites. Further by optimizing the interval between doses we successfully elicited long-term increases in CD4+ T cell levels. Therefore these studies provide further evidence that IL-7 can be used effectively as an immunotherapeutic to increase the absolute number of CD4+ T cells to assist in the recovery of the immune system dysfunction induced by the SIV or HIV infections.

Table 3-1: Absolute T cells/mL of blood in uninfected and SIV+ macaques prior to rsIL-7gly.

	Pre-rsIL-7gly CD4+ T cell Levels			*Pre-rsIL-7gly CD8+ T cell Levels*		
	Total	*Naïve*	*CM*	*Total*	*Naïve*	*CM*
Average Levels in 8 Uninfected Macaques	962.14	640.73	211.14	498.7	256.57	5.75
rsIL-7gly dosing interval study						
RM2-01	863.11	535.93	188.10	730.90	331.60	37.49
RM2-02	899.21	454.50	210.09	836.88	225.44	23.22
RM2-03	564.89	340.90	146.72	635.96	221.53	32.79
RM2-04	1054.71	403.29	340.12	1633.35	355.89	92.75
RM2-05	665.58	383.13	161.67	662.30	146.10	19.76
RM2-06	519.35	285.78	149.23	1347.99	234.05	38.19
rsIL-7gly Dosing at 6-week Intervals						
RM2-01	595.70	318.56	125.84	610.35	96.26	31.16
RM2-03	469.04	245.15	115.86	547.27	98.30	23.61
RM2-04	987.36	398.56	318.91	1627.83	209.24	64.87
rsIL-7gly clustered dosing						
RM2-05	728.45	401.50	149.85	568.73	161.43	13.71
RM2-07	955.59	543.38	241.46	624.31	179.51	19.60

TABLE 3-2- Effect of rsIL-7gly administration on SIV plasma viral load

Monkey number	-7 d	7d	14d	21d	28d	63d	77d	105d
rsIL-7gly 2, 4 or 6 week Intervals								
RM2-01	60	490	240	340	150	110	70	-
RM2-02	30	340	30	30	30	30	40	-
RM2-03	740	620	590	440	340	320	200	-
RM2-04	50	40	30	150	30	40	50	-
RM2-05	30	260	120	50	30	30	30	-
RM2-06	8800	1500	4300	4400	3700	6500	1300	-
rsIL-7gly 6 week Intervals								
RM2-01	190	490	70	80	30	810	30	70
RM2-03	310	40	140	230	330	250	110	70
RM2-04	30	90	170	30	30	40	50	30
Clustered rsIL-7gly Doses								
RM2-05	100	30	120	110	30	30	30	30
RM2-07	30	70	50	30	-	1000	1000	3800

TABLE 3-2- Plasma viral load (SIVgag copies eq/mL) was monitored over the course of rsIL-7gly administration in all the cohorts using a real time PCR based assay. The limit of detection was 30copies/mL.

Figure 3-1: rsIL-7gly induces robust proliferation in uninfected and SIV+ macaques

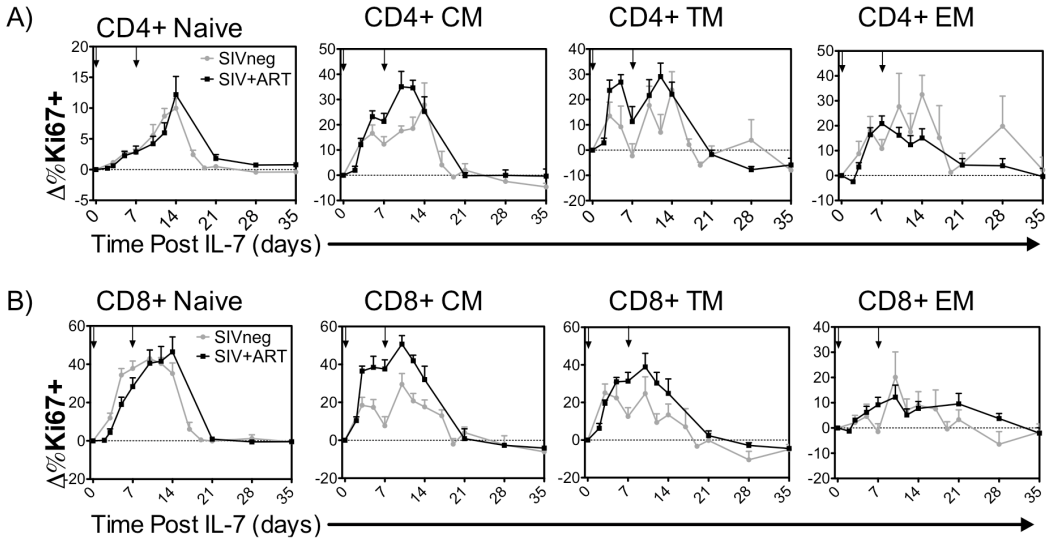


Figure 3-1: rsIL-7gly induces robust proliferation in SVneg and SIVpos Rhesus macaques:
rsIL-7gly was administered where indicated [↓]. The nuclear antigen Ki67 was used to assess proliferating cells, pre-rsIL-7gly levels of proliferation were determined by averaging the percentage of Ki67+ cells in each subset over three timepoints. The difference in Ki67 expression from pre-rsIL-7gly levels was determined for each subsequent timepoint and is presented as the mean±standard error of the mean (SEM). The dashed horizontal line indicates pre-rsIL-7gly Ki67 levels. (A) Change in percent Ki67+ cells in CD4+ subsets; (B) change in percent Ki67+ cells in CD8+ subsets. Uninfected macaques (N=8; grey), SIV+ART treated macaques (N=6; black)

Figure 3-2: rsIL-7gly increases T cell levels in uninfected and SIV+ macaques

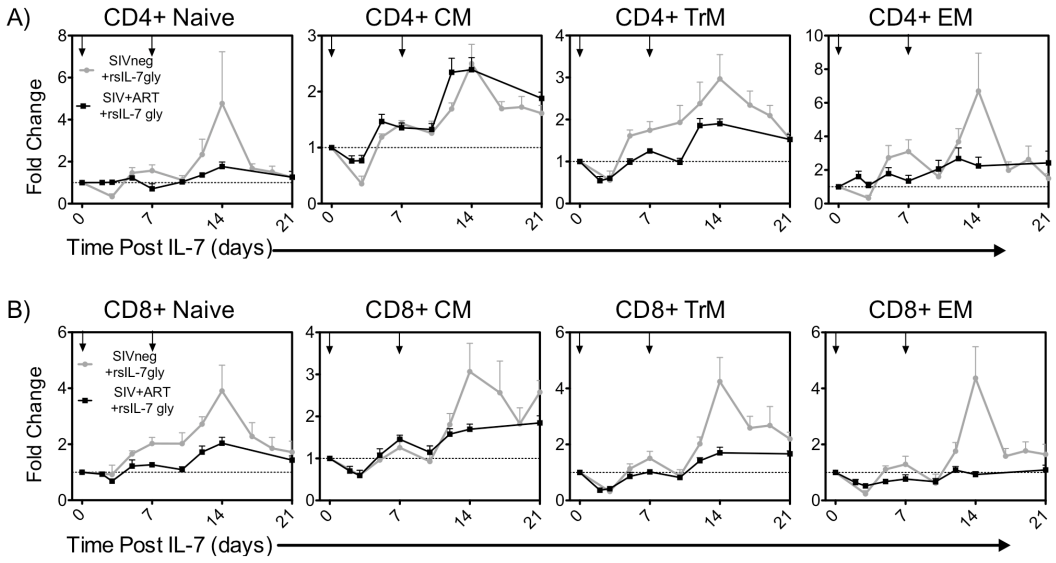


Figure 3-2: Change in absolute T-cell number in response to rsIL-7gly in uninfected and SIV+ART treated macaques:
rsIL-7gly was administered where indicated [↓]. Pre-rsIL-7gly T-cell levels were determined by averaging the number cells in each subset over three timepoints. The fold change from baseline was determined for each subsequent timepoint. The dashed horizontal line indicates pre-rsIL-7gly levels. The data is presented as the mean±SEM. (A) Fold change in CD4+ subsets; (B) Fold change in CD8+ subsets. Uninfected macaques (N=8; grey), SIV+ART treated macaques (N=6; black).

Figure 3-3: Administration of rsIL-7gly induces maximal Ki67 responses at 4-6 week intervals

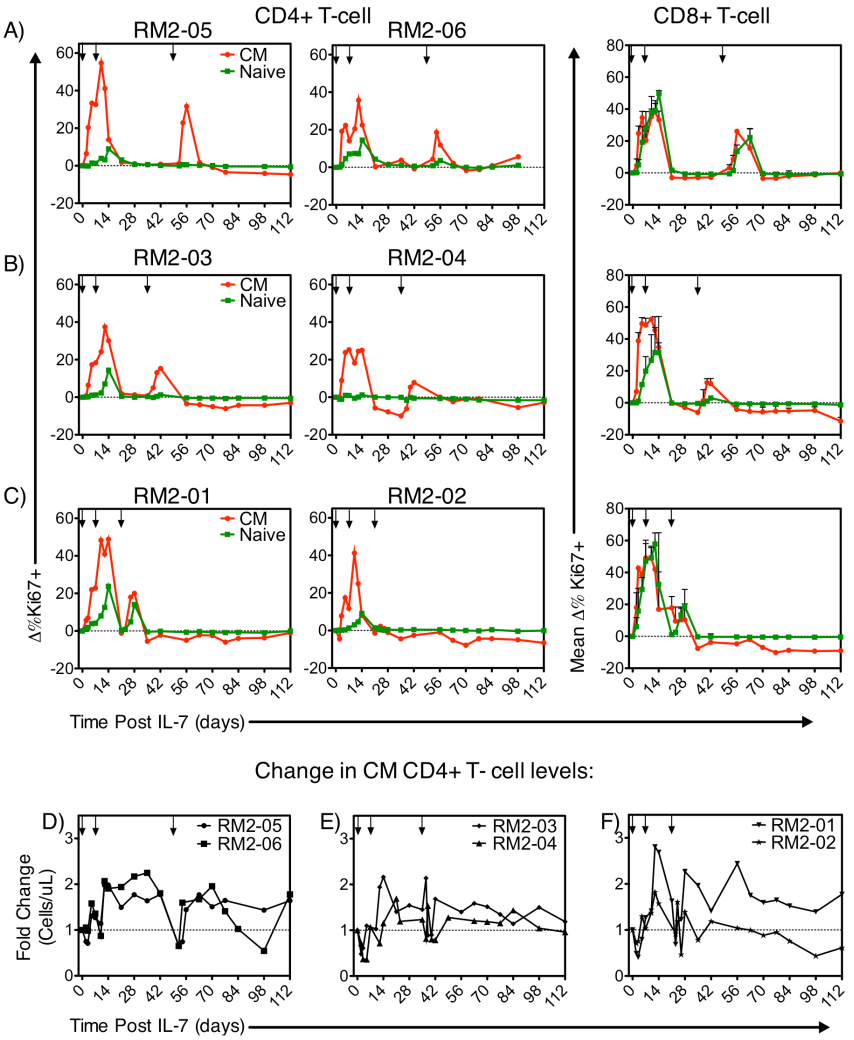


Figure 3-3: Increased Ki67 expression patterns for determining rsIL-7gly dosing Intervals. rsIL-7gly induced proliferation (Ki67+) cells was assessed by flow cytometry at 2-week intervals in groups of 2 macaques. The change from baseline for CM (red) and naïve (green) cells was calculated for each subsequent point. rsIL-7gly administration is indicated by [↓]. (A) Change in percent Ki67+ cells in RM2-05 and RM 2-06. (B) Change in percent Ki67+ cells in RM2-03 and RM 2-04 (C) Change in percent Ki67+ cells in RM2-01 and RM 2-02. The fold change was also determined for the absolute number of CD4+ CM T cells in each of the macaques. (D) Percent change in RM2-05 and RM2-06 (E) percent change in RM2-03 and RM2-04 (F) percent change in RM2-01 and RM2-02.

Figure 3-4: Administration of rsIL-7gly at 6-week intervals transiently increases T cell proliferation and number in SIV+ ART treated macaques

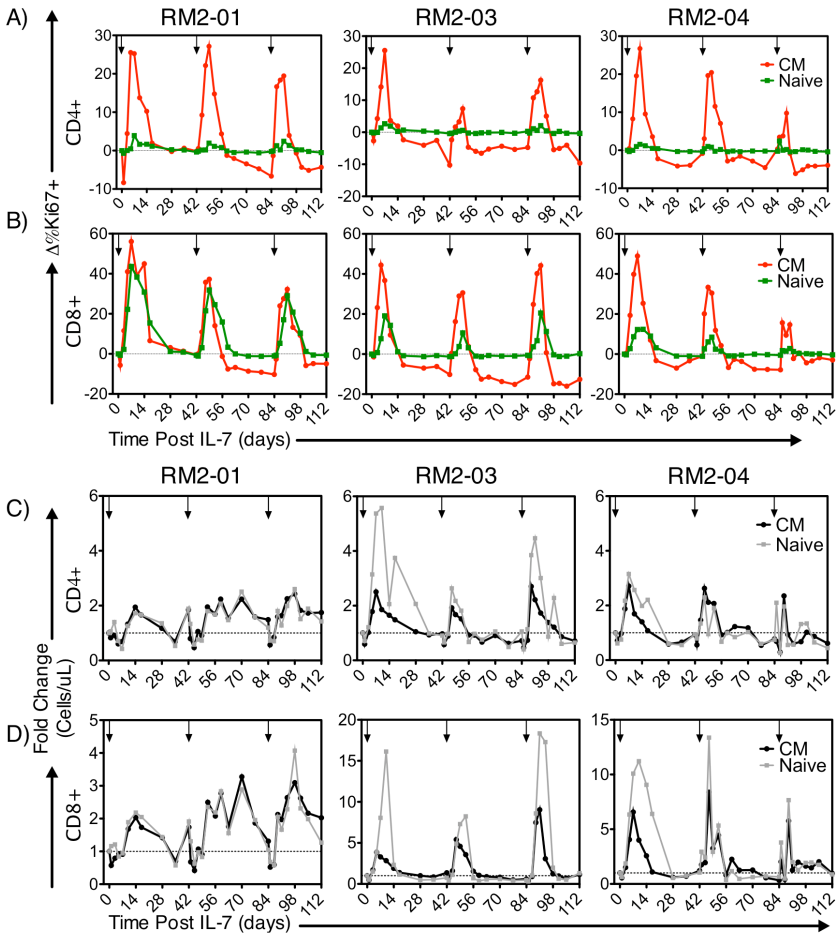


Figure 3-4: IL-7 administration during fully controlled SIV infection at 6 week intervals transiently increase T-cell number and proliferation.
We assessed the change in Ki67+ T cells from pre-grm-IL-7 levels at each time point. Basal Ki67 levels were determined by averaging three rsIL-7gly time points. rsIL-7gly administration is indicated by [↓]. CM (red); naïve (green). (A) CD4+ T cells (B) CD8+ T cells. We then determined the effect of rsIL-7gly on the absolute number of T cells over time. The fold change was determined from the basal level of each T-cell subset; CM (black); naïve cells (grey). (C) Absolute number of CD4+ T cells (D) Absolute number of CD8+ T cells

Figure 3-5: Administering clustered doses of rsIL-7gly transiently increases T cell proliferation in ART treated SIV+ macaques

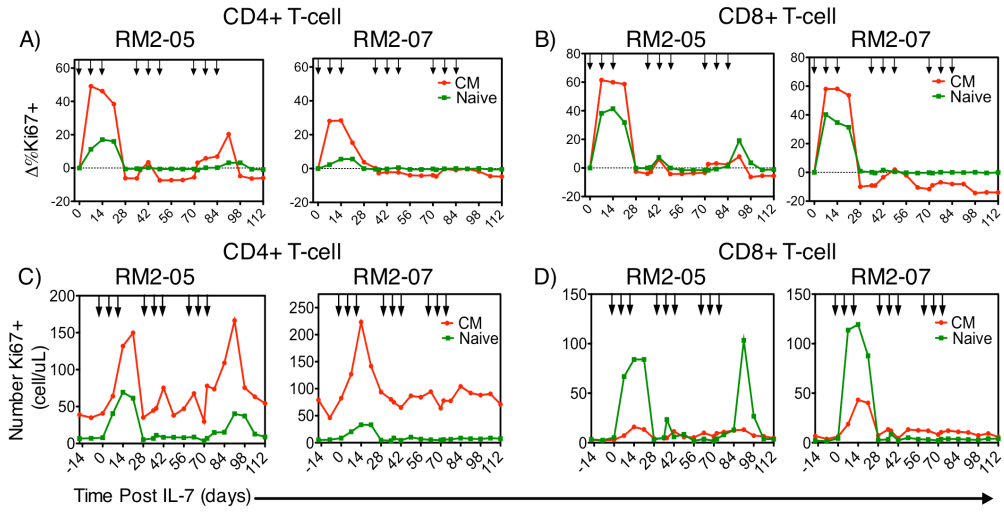


Figure 3-5: Three clustered doses of rsIL-7gly increases proliferation in peripheral T cells. Two macaques treated with stable ART therapy were administered rsIL-7gly at weekly intervals for three weeks followed by a three-week wash out period. Proliferation in peripheral T-cell subsets was monitored by flow cytometry assessing the fraction of Ki67+ cells. Each rsIL-7gly dose is indicated by [↓]. The change in Ki67 expression from baseline levels is shown. CM (red); green (naïve) (A) CD4+ T cells (B) CD8+ T cells. We also determined the absolute number of proliferating cells in each macaque in each subset (C) CD4+ Tcells (D) CD8+ T cells.

Figure 3-6: Administering clustered doses of rsIL-7gly increases T cell number in ART treated SIV+ macaques

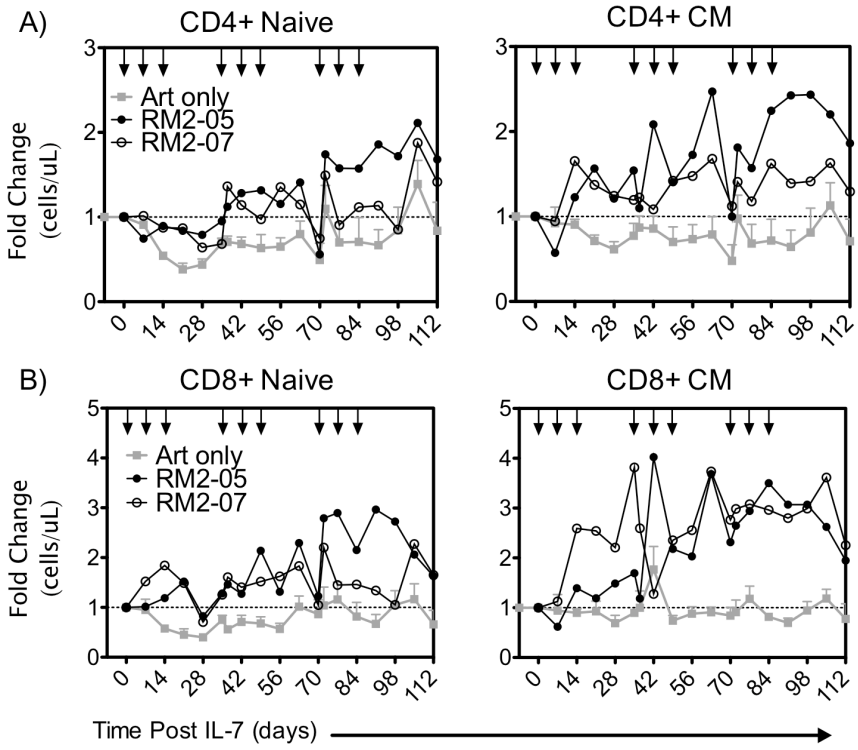


Figure 3-6: rsIL-7gly administration results in long-term increases in absolute T-cell number during fully controlled SIV infection.
Where applicable rsIL-7gly administration is indicated [↓]. The mean absolute T-cell count of four macaques treated with ART alone (mean ± SEM) (grey square; 1*102 copies/mL <VL < 1*106 copies/mL) as well as RM2-05 (closed circle) and RM2-07 (open circle) is shown in each figure. (A) Percent change in CD4+ T-cell subsets (B) percent change in CD8+ subsets

Figure 3-7: rsIL-7gly increases the half-life of BrDU labeled cells in the peripheral blood

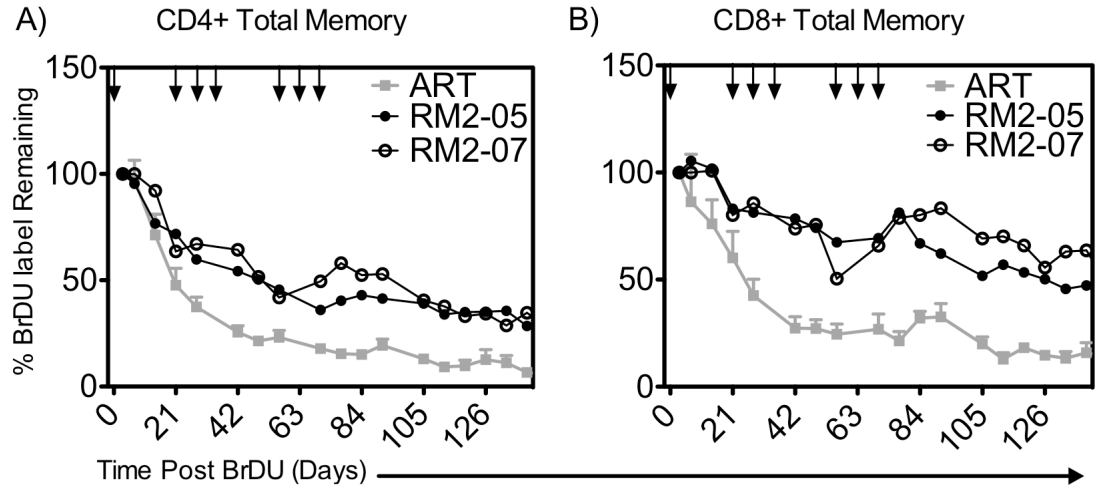


Figure 3-7: rsIL-7gly induces increases the percentage of BrDU+ cells in tissues and increases the half-life of labeled cells

BrDU was used to label cells dividing in response to rsIL-7gly administration following the first of nine doses. The percentage of BrDU+ cell immediately post labeling was set to 100% to normalize the rate of decline in all the macaques. Where applicable rsIL-7gly administration is indicated [↓]. ART controls (mean +/- SEM) (grey square) as well as RM2-05 (closed circle) and RM2-07 (open circle) are shown. The percentage of BrDU label retained in CD4+ memory cells in the peripheral blood (B) The percentage of BrDU label retained in CD8+ memory cells in the peripheral blood

CHAPTER FOUR

IL-7 TREATMENT IN ART TREATED SIV+ MACAQUES INCREASES NAÏVE B CELL LEVELS BUT NOT CD3+CD4-CD8- T CELLS

Introduction and Rationale:

While the primary immune system damage from an SIV infection in macaques is depletion of the CD4+ T cells, a second source of immune system damage is aberrantly high levels of immune activation (75, 123, 258). Clinically, HIV+ patients are particularly prone to opportunistic infections including pneumocystis carinii, candidiasis and cytomegalovirus (Morbidity and Mortality Weekly Report 2004 No. RR-15). In addition, vaccine responses in HIV+ patients are attenuated (79, 101, 185, 241, 269, 278) making it difficult to protect these patients from common co-infections with approved vaccines such as influenza [Morbidity and Mortality Weekly Report 2004 No. RR-15] and tuberculosis [Morbidity and Mortality Weekly Report 2004 No. RR-15]. As part of the ongoing work in our laboratory, we have begun to examine the dysfunction in alternate immune cell subsets that may be crucial to host defense.

CD3+CD4-CD8-pangd- (CD3+CD4-CD8-/double negative) T cells are an alphabeta T cell subset that has not been studied as closely as either single positive T cells or B cells. They are usually extrathymic in origin and are thought to originate as activated single positive T cells that lose expression of either the

CD4 or CD8 co-receptor (86, 276). Studies in mice have establish a role for these cells in peripheral tolerance although they have been implicated in anti-tumor activity (299), prevention of graft versus host disease (GVHD) in allogenic bone marrow transplants (85) and as a potential regulatory T-cell subset (48, 86, 276). CD3+CD4-CD8- T cells have cytotoxic properties directly killing activated CD4+ and CD8+ T cells via Fas/FasL interaction in a contact dependent manner (299) generally targeting single positive T cells with the same TCR antigen specificity (276). As part of their role in the contraction phase of the immune response, double negative T cells acquire Ag-MHC complexes from APCs to kill antigen specific T cell clones (48, 276). The effect of HIV infection on their number and proliferation is imprecisely defined in humans, though the percentage of CD3+CD4-CD8- cells has been shown to increase with infection (168). Studies in natural SIV hosts including African green monkeys and sooty mangabeys, indicate that in non-pathogenic models double negative T cells potentially retain T-helper cell functionality and are resistant to SIV infection (Sodora unpublished, (17)). Therefore, the potential for these SIV-resistant multifunctional T cells to respond to a cytokine immune therapy such as IL-7 was of particular interest to our laboratory.

B cell studies have documented dysfunction in naïve and memory B cells during HIV infection with dysfunctions including: hypergammaglobulinemia [reviewed

in (178)], depletion of B cell number (186), qualitative changes in the phenotype of the cells (eg naïve and memory or activation state) (42, 59, 180, 181, 186, 278), diminished B cell functional responses (118, 141, 164, 179, 278), reduced vaccine effiacay (79, 101, 185, 241, 269) and increased susceptibility to apoptosis (181). Some of these dysfunctions are reparable with effective antiviral treatment (164, 180, 181) though memory B cells remain depleted throughout the chronic phase of an HIV infection (59). In collaboration with Dr. Kelly Cole at the University of Pittsburgh and her student, David Kuhrt, we undertook a longitudinal study examining B-cell dynamics during acute SIV infection prior to the initiation of ART and rsIL-7gly treatment that has been submitted for publication (Journal of Virology). These studies indicated an immediate and precipitous decline in the memory B cell population. We hypothesized that rsIL-7gly administration would benefit the B cell compartment in ways similar to the effect on T cells, increasing cell number in both naïve and memory subsets.

Results:

Viral load variation during acute SIV infection in 10 Rhesus Macaques distinguished three levels of viral control:

One of the secondary aims of our study was to determine whether rsIL-7gly administration was beneficial to overall immune system health by ascertaining its effect on other immune cell populations, for this we chose to investigate CD3+CD4-CD8- T cells as well as B cells cells. To address this question the impact of rsIL-7gly administration on both CD3+CD4-CD8- number and B cells during stable ART treatment. A varying degree of viral control was observed during acute infection in our cohort of ten macaques enrolled in the rsIL-7gly clustered dosing regimen (RM2-02, RM2-05, RM2-06, RM2-07, RM2-08, RM2-09, RM2-10, RM2-11, RM2-12 and RM2-13) (Figure 4-1). Three macaques RM2-02, RM2-05 and RM2-07 were able to spontaneously reduce viral replication and developed set point plasma viremia ranging from 1*103 to 1*105 copies/ml (Figure 4-1). This set point was at least two logs lower than the macaques with the highest set points (RM2-08, RM2-12 and RM2-13) that ranged from 1*107 to 1*108 copies/ml (Figure 4-1). SIV vaccine studies that elicit a similar reduction in viral load have demonstrated that this is sufficient to attenuate the rate of disease progression (14, 43, 109, 119, 124, 134, 265).

CD3+CD4-CD8- T cells in peripheral blood of Rhesus macaques

In our macaque model we identified the CD3+CD4-CD8- T cells from macaque peripheral blood cells as shown in Figure 4-2. Briefly, to ensure the cleanest identification of double negative T cells, CD20+ B cells were excluded from the analysis, as were CD3+CD4+ T cells. Within the CD3+CD4- subset there are three possible populations of cells: CD3+CD8+ single positive T cells, CD3+CD8intermediatepangd+ and CD3+CD8-pangd- T cells. To distinguish these populations, the pangd+ T cells were excluded from the analysis and only CD8- cells were included in the double negative T cell population. This gating strategy ensured minimal contamination of the double negative population by either pangd+ or single positive CD8+ T cells (Figure 4-2).

During Acute Infection CD3+CD4-CD8- proliferation correlates with reduced viral set point

Peak viremia in these macaques occurred approximately 14 days post infection and was associated with a decreased percentage of proliferating CD3+CD4-CD8- cells in the peripheral blood (Figure 4-3A). Following the peak in viral replication, the macaques with low viral set point (N=3) display an increase in proliferation in the CD3+CD4-CD8- cells that remains elevated (range= 10.1% to 20.3% at day 98) through the first 100 days of infection (Figure 4-3A). Macaques with high viral set points (N=3) fail to increase the percentage of proliferating CD3+CD4-CD8- cells from its nadir point and remain suppressed through 100 days post-infection (Figure 4-3A). It was therefore not surprising that the

macaques in the mid set point group (N=4) increase the percentage of proliferating CD3+CD4-CD8-Ki67+ cells above baseline levels as viral set point but to an intermediate level between the low and high viral set point groups (range= -7% to 12% at day 98) (Figure 4-3A). In addition, a modest inverse correlation (R2 = 0.27) was observed between CD3+CD4-CD8- proliferation and set point viremia (Figure 4-3B). Furthermore, this increase in proliferation in the low set point group did not translate into an increase in the absolute level of CD3+CD4-CD8- cells which remained relatively constant over the 100 days post-infection for all three viral set point groups (Figure 4-3C).

Effect of rsIL-7gly administration on CD3+CD4-CD8-s during ART controlled chronic infection

Following acute infection, the macaques were placed on ART consisting of PMPA (30mg/kg/d) and FTC (50mg/kg/d) (see materials and methods). Two macaques with fully suppressed viral replication on ART (RM2-05 and RM2-07) were given three dose clusters of rsIL-7gly at weekly intervals followed by a three-week washout period (Table 2-1). This regimen was repeated three times. Four macaques were used as control animals and received only ART treatment (RM2-02, RM2-06, RM2-10 and RM2-11) (Table 2-1). In contrast to the CD4 and CD8 T cells assessed in Chapter 3, the rsIL-7gly did not elicit any increase in CD3+CD4-CD8- proliferation (Figure 4-4A). In addition, rsIL-7gly did not alter the absolute number of circulating CD3+CD4-CD8- cells in the peripheral blood

(Figure 4-4B). These results indicate that this regimen of rsIL-7gly therapy, which appeared functional for CD4+ and CD8+ T cells, did not influence CD3+CD4-CD8- proliferative abilities or peripheral blood levels of these SIV+ ART treated macaques .

Effect of rsIL-7gly administration on naïve and memory B cells during ART controlled chronic infection

Given the role of IL-7 in de novo production and maintenance of B cells in the murine system (6, 83, 174, 203) we sought to assess whether rsIL-7gly administration in primates would be able to increase the number of B cells during chronic infection. We were able to phenotypically subdivide the B cells based on the expression of surface markers in-vivo (naïve: CD20+ CD27- and memory: CD20+ CD27+). rsIL-7gly administration induced minimal proliferation in both naïve (Figure 4-5A) and memory (Figure 4-5B) B cells in RM2-05 and RM2-07. Therefore it was surprising that rsIL-7gly administration did increase the absolute number of naïve B cells in these SIV+ ART treated macaques (Figure 4-6A), although the absolute number of memory B cells was not affected (Figure 4-6B). It is possible that the rsIL-7gly induced naïve cell proliferation could in turn influence the levels of SIV-envelope specific antibody titers. Plasma antibody titers were measured at day 126-post infection (prior to rsIL-7gly in treated macaques) and day 233-post infection (post rsIL-7gly in treated macaques) in both rsIL-7gly treated and untreated cohorts. Macaques in both cohorts increased

their plasma antibody titer over time (Figure 4-6C). However, the change in antibody titer was not significant in the ART suppressed control macaques that were not given rsIL-7gly ($p=0.6631$), but was significant in our two macaques ($p < 0.0001$) in the rsIL-7gly treated ART suppressed cohort (Figure 4-6C). These results suggest rsIL-7gly administration was associated with increased anti-SIV antibodies in addition to increasing naïve cell numbers.

Discussion:

HIV/SIV infection is associated with depletion of CD4+ T cells during an aggressive acute infection and over time (36, 151, 169, 219). In the studies presented in this dissertation, our primary objective was to assess whether rsIL-7gly therapy was able to reconstitute the damage to the CD4+ T-cell compartment. However, our laboratory also has a long-standing interest in the function and maintenance of alternative immune cells stemming from studies in the Sodora laboratory as well as collaborators. Two cell types that we are particularly interested in are CD3+CD4-CD8- double negative T cells and B cells which are discussed in this chapter.

Do CD3+CD4-CD8-s Appear to Associate with Severity of Acute Infection?

These studies examined the dynamics of the CD3+CD4-CD8- population longitudinally during acute infection and suggest that there could be an inverse

correlation between CD3+CD4-CD8- proliferation and established set point viremia (Figure 4-3B). SIV+ macaques in the highest set point cohort displayed CD3+CD4-CD8- proliferation that remained below pre-infection levels for the duration of the acute infection (Figure 4-3A). One potential explanation of this association is that the CD3+CD4-CD8- proliferative capacity is important for containing/controlling viral replication in this model. It was surprising that despite the elevated levels of Ki67 expression in the low-set point macaque cohort, there was no net increase in CD3+CD4-CD8- numbers in the blood of macaques that controlled virus replication (Figure 4-3C). This could be due to any number of factors including that possibility that the newly created CD3+CD4-CD8- T cells are trafficking to tissues other than the peripheral blood. CD3+CD4-CD8- cells may be able to contribute to shaping the dynamics of virus replication in several ways. They have been shown to serve as a viral reservoir (121) suggesting they may contribute to increasing latent virus. However, it is more likely that the immunosuppressive properties of CD3+CD4-CD8- T cells contribute to reducing the pool of activated SIV target cells. CD3+CD4-CD8- cells also have documented anti-viral properties in a murine cytomegalovirus system (MCMV) system (110) which speculatively suggests that CD3+CD4-CD8- double negative T cells may have anti-SIV properties as well as possibly reducing SIV target cells. In addition, a recent study by Beaumier et al (17)

indicates that these cells may retain sufficient helper function to be able to compensate for the depleted CD4+ T cells.

IL-7 and B cell development

In the murine model, IL-7 is critical for the de novo production and peripheral survival of B cells (6, 83, 174, 203). The role of IL-7 in B-cell development was originally identified in IL-7 deficient mice that showed a reduced number of mature B cells (218, 286). During HIV infection, studies by our collaborators [D. Kuhrt submitted AIDS research and Human Retroviruses (140)] and others (278) have demonstrated that acute infection is critical for the establishing the dysfunction in B cells. The results of these studies indicate there is a precipitous decline in the absolute number of B cells rapidly following infection decreasing within the ten animal cohort from a mean of 1157cell/mL of blood to 378 cells/mL within the first ten days of infection (140). However, unlike CD4+ T cells, the absolute number of CD20+ B cells regained pre-infection levels in the absence of ART by 80-90 days post infection (140). Interestingly, similar to CD4+ T cells, the dynamics of B cell subsets, naïve, IgG/IgA or IgM secreting memory cells were differentially impacted by SIV infection (140). All the subsets sustained severe depletion within the first 10 days, however, the IgG/IgA secreting memory and naïve (CD20+CD27-) B cells recovered pre-infection

levels in the absence of ART while the IgM secreting memory cells remained significantly depleted (140). One possible reason for the failure of IgM secreting memory cells to regain pre-infection levels is that the percentage of Ki67+ cells in this population slowly declined over the course of infection in contrast to IgG/IgA memory cells which were able to significantly increase proliferation following their depletion (140). It is possible to speculate that the shift in memory B cell population toward IgG/IgA secreting cells away from IgM secreting cells which have been shown to be critical for antibody production in response to viral mutants and opportunistic infections may increase the immunocompromised status of the host. Endogenous IL-7 levels during chronic HIV have been inversely correlated with B cell dysfunction (high plasma IL-7 correlates with low function) in clinical studies (164) though a causative role for high levels of IL-7 in B cell dysfunction has not been identified. ART treatment in our cohort was partially successful in restoring the B cell populations to 'normal' levels, initiating a transient increase in naïve cells (408 cells/mL when ART initiated peaking at 562 cells/mL) and a sustained increase in memory B cells (571 cells/mL to 954 cells/mL) (140). However, the IgM secreting population showed no increase in absolute cell number indicating that the IgG/IgA memory subset underwent the bulk of the expansion (140). We hypothesized that rsIL-7gly therapy may be able to help normalize particularly the naïve B cell subset when administered in conjunction with successful ART.

Effect of rsIL-7gly Administration on CD3+CD4-CD8-s and B cells:

rsIL-7gly was administered to two macaques in three dose clusters (Table 2-1) during chronic ART controlled infection. In contrast to our hypothesis, rsIL-7gly had negligible impact on CD3+CD4-CD8- proliferation and cell number in the blood (Figure 4-4). An impact on these parameters outside the blood at secondary sites cannot however be discounted. In addition, rsIL-7gly administration did not elicit increased Ki67 expression in naïve or memory B cells when viral replication is fully suppressed (Figure 4-5A and 4-5B). However, the absolute number of peripheral blood naive B cells did increase in response to rsIL-7gly treatment (Figure 4-6A). Memory B cells remained at levels comparable to ART controls (Figure 4-6B). This suggests that rsIL-7gly may be able to elicit increased production of de novo B cells. rsIL-7gly treatment may also increase the titer of anti-SIV antibodies (Env specific) (Figure 4-6C). The sum of these observations suggests that rsIL-7gly may be able to increase both naïve B-cell number and antibody production when SIV infection is controlled with ART.

Summary:

rsIL-7gly administration appears to have limited impact on double negative T cells. The ability of rsIL-7gly to increase the levels of naïve B cells could

potentially provide clinical benefit by increasing resistance to OIs, and this should be investigated in future IL-7 therapy trials in HIV+ humans. However, overall rsIL-7gly appears to have the most potential as a CD4+ targeted immunotherapy.

Figure 4-1: Plasma viral load during acute SIV infection in 10 SIVmac239 infected rhesus macaques

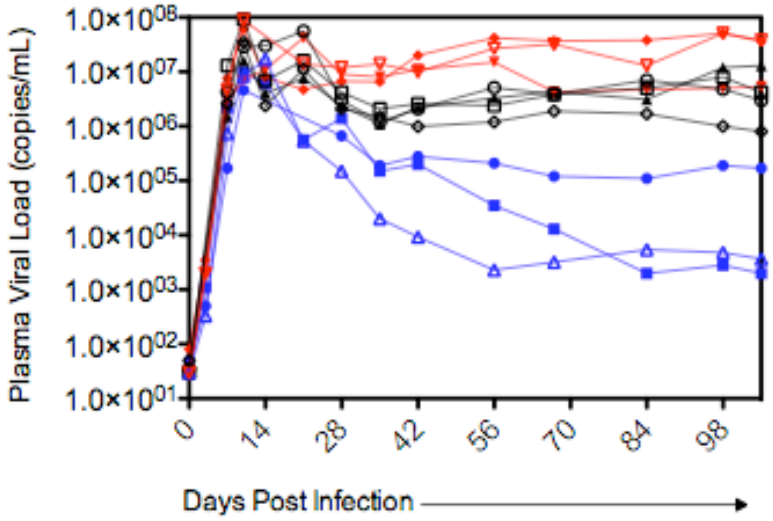


Figure 4-1: Plasma viral load during acute SIV infection. Ten Indian origin rhesus macaques were intravenously inoculated with SIVmac239. The number of SIVgag copies/mL was quantified over the first 105 days of infection to measure the viral load in the plasma. Three macaques exhibited a low viral set point below 105 copies/mL (Blue lines; RM-2-02, RM2-05 and RM2-07). Four macaques displayed viral loads between 106-107 copies/mL (Black lines; RM2-10, RM2-06, RM2-09 and RM2-11) and three macaques had viral loads above 107 (Red lines; RM2-08, RM2-12 and RM2-13).

Figure 4-2: Gating strategy to identify CD3+CD4-CD8- T cells

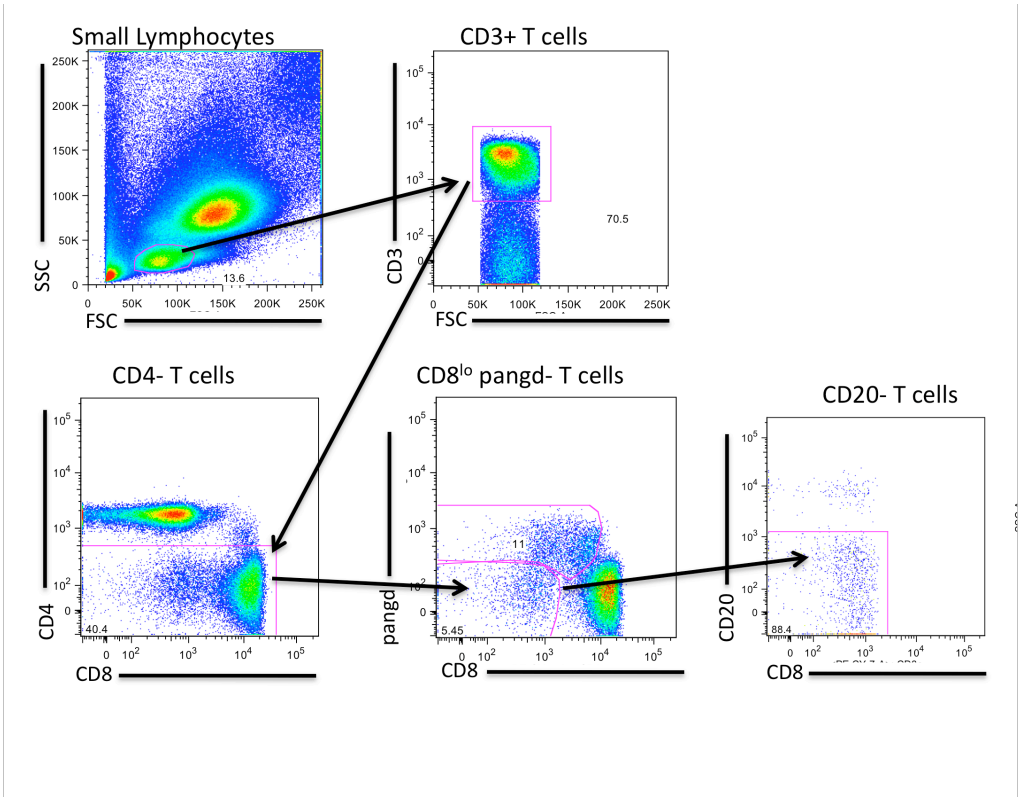


Figure 4-2: Gating strategy to identify CD3+CD4-CD8- T cells. Small lymphocyte were identified using the forward and side scatter parameters. CD3- lymphocytes were then excluded as were CD4+ T cells. To cleanly identify double negative cells, CD8high and pangd+ Tcells were then separated. The final step was to ensure that CD20- B cells were excluded from the final analysis.

Figure 4-3: Double negative T cell number and proliferation during acute infection may be associated with viral load

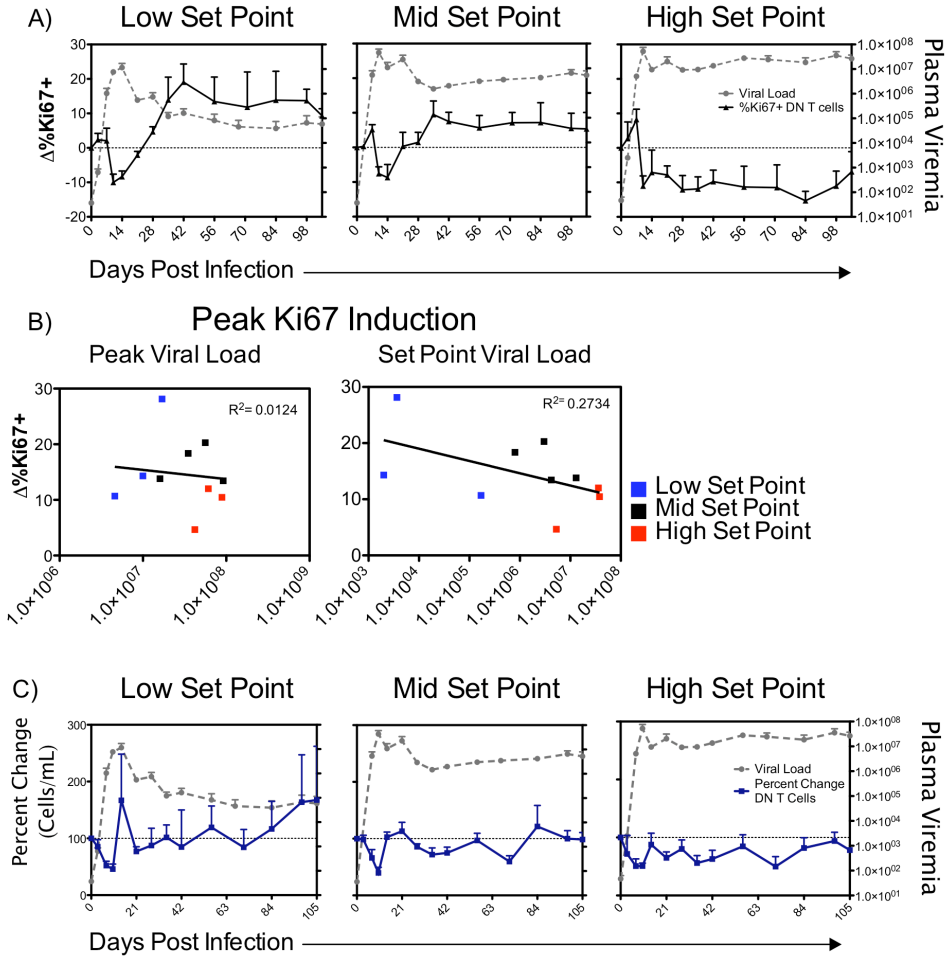


Figure 4-3: CD3+CD4-CD8- number and proliferation during acute infection may associate with viral load. Using flow cytometry we assessed the number of CD3+CD4-CD8- T cells over acute infection. The nuclear antigen Ki67 was used as a marker for proliferation. The mean±SEM is shown for each group. The difference in Ki67 expression was determined from baseline (average of three pre-infection timepoints). Left Y-axis; Mean±SEM CD3+CD4-CD8- proliferation or absolute number (black line) Right Y-axis; Mean±SEM viral load for each group (grey line). (A) Change in CD3+CD4-CD8- proliferation (B) Correlation between peak CD3+CD4-CD8- proliferation and viral loads. (C) Left Y-axis; Mean±SEM Fold change in CD3+CD4-CD8- number (blue line) Right Y-axis; Mean±SEM viral load for each group (grey line).

Figure 4-4: rsIL-7gly administration does not induce proliferation in circulating CD3+CD4-CD8- T cells

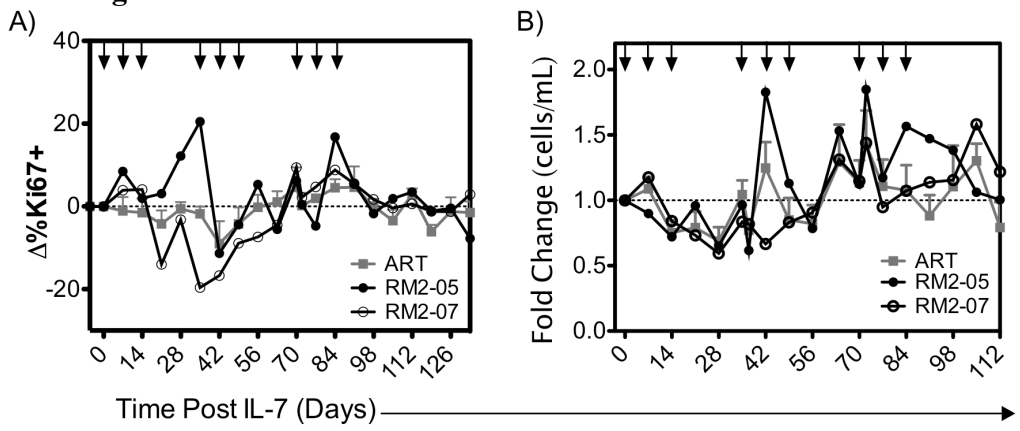


Figure 4-4: rsIL-7gly administration does not alter CD3+CD4-CD8- proliferation or number during ART controlled SIV infection. To assess the impact of rsIL-7gly on CD3+CD4-CD8-s the cytokine was administered where indicated [↓]. Proliferation was assessed using the nuclear antigen Ki67. ART control animals are shown as the cohort mean and standard error of the mean (grey line). (A) Change in percent Ki67+ CD3+CD4-CD8- in RM2-05 (black closed circle) and RM2-07 (black open circle). (B) Fold change in absolute cell number in RM2-05 (black closed circle) and RM2-07 (black open circle)

Figure 4-5: Effect of rsIL-7gly administration on proliferation in peripheral B cells

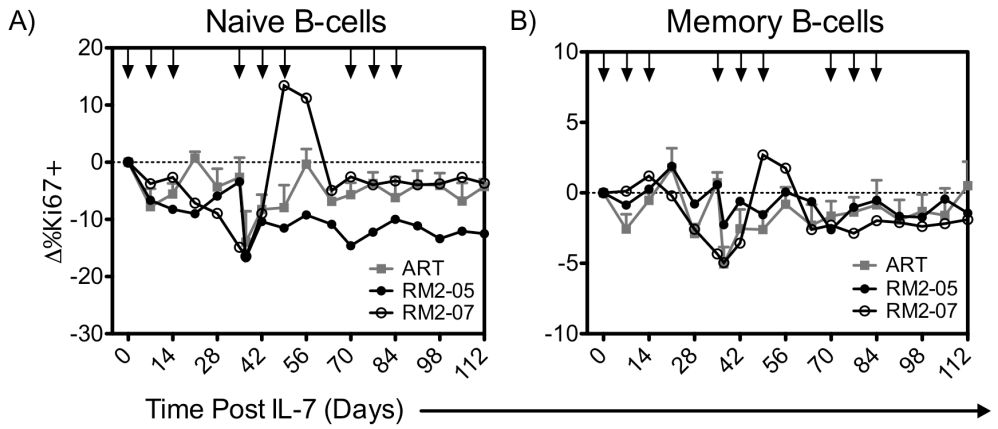


Figure 4-5: rsIL-7gly administration Induces minimal proliferation in peripheral B cells. rsIL-7gly was administered where indicated [↓] in three dose clusters spaced three weeks apart. Ki67 was used to measure proliferation in naïve and memory B cells circulating in the periphery. ART treated control animals are shown as the cohort mean +/- SEM (grey line). (A) Change in percent Ki67+ naïve B cells in RM2-05 (black closed circle) and RM2-07 (black open circle). (B) Change in %Ki67+ memory B cells in RM2-05 (closed circle) and RM2-07 (open circle)

Figure 4-6: Effect of rsIL-7gly administration on the absolute number of peripheral B cells

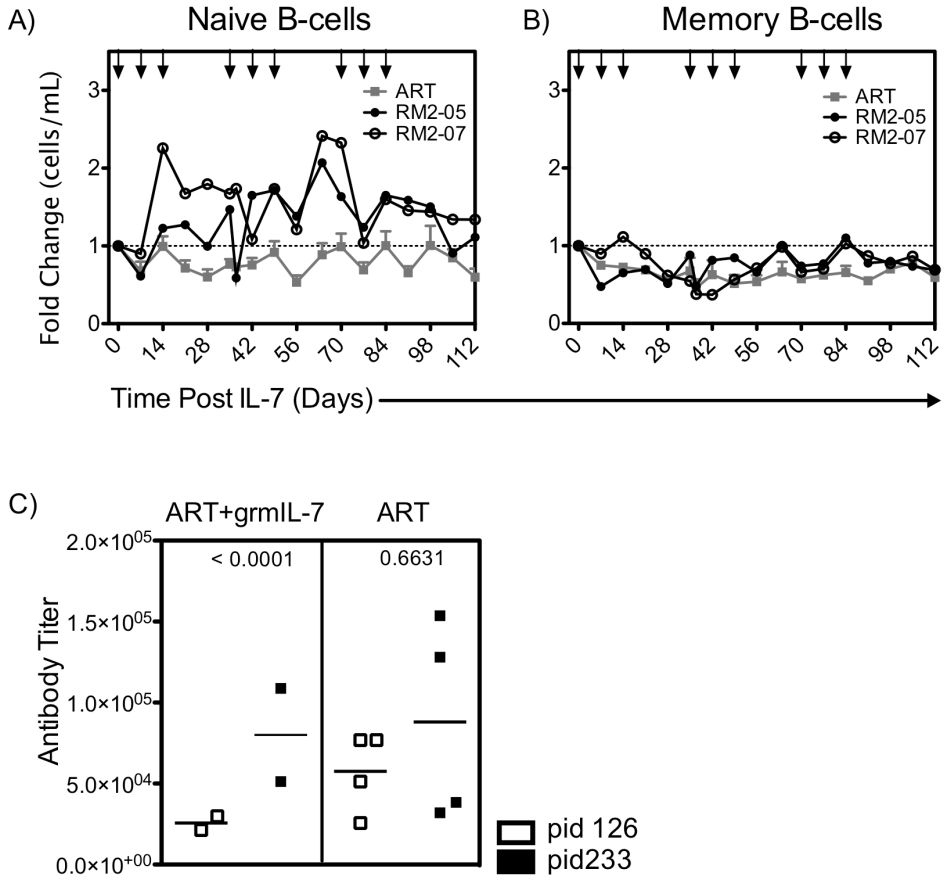


Figure 4-6: rsIL-7gly Administration Increases the absolute number of naïve but not memory B cells. rsIL-7gly was administered where indicated [↓] in three dose clusters spaced three weeks apart. Flow cytometry was used to monitor the number of naïve and memory B cells circulating in the periphery. (A) Fold change in absolute naïve B cells (grey line; ART treated controls; closed circle; RM2-05 and open circle RM2-07). (B) Fold change in absolute memory B cells (grey line; ART treated controls; closed circle; RM2-05 and open circle RM2-07). (C) Reciprocal plasma titer of anti-SIV envelope specific antibodies pre and post rsIL-7gly treatment. Significance was calculated using the Mann-Whitney T-test and exact p values are shown.

CHAPTER FIVE

ASSESSING THE IMPACT OF SIV INFECTION OF IL-7 FUNCTION IN-VIVO IN THE SIV+ RHESUS MACAQUE MODEL

Introduction and Rationale:

The studies presented in chapter 3 and 4 unexpectedly generated several anecdotal lines of evidence that active SIV infection impaired the response of peripheral T cells to rsIL-7gly administration. We hypothesized that during untreated infection, the loss of IL-7 function in-vivo may be contributing to the failure of the central memory T cell compartment. Dysfunction in the IL-7 signaling axis has been documented as HIV/SIV has been shown to decrease IL-7R (CD127) expression and cause dysfunction in the activation of downstream signaling intermediates (1, 22, 23, 27, 28, 45, 87, 131, 132, 139, 171, 173, 183, 200, 211, 212, 216, 233, 242, 253, 291, 300). HAART is able to partially restore the IL-7/IL-7R axis (53, 54, 162, 230).

Overview of IL-7 Signaling Pathways:

Cytokine signaling is canonically associated with three intertwined signaling pathways, JAK/STAT, PI3K and MAPK. JAK/STAT signaling downstream of the IL-7R (Figure 5-1) has been extensively studied and is reviewed comprehensively by Jiang et al (116). JAK3 is constitutively associated with the

common gamma chain (CD132) and upon dimerization with the IL-7 specific alpha chain (CD127) phosphosrylates tyrosine 449 (Y449) creating a docking site for downstream molecules including STAT5 and PI3K (116). JAK3 also phosphorylates STAT family proteins, predominately STAT5 in IL7 mediated signaling, which form dimers and larger complexes that are translocated to the nucleus to initiate signaling (20, 116, 122, 259). JAK/STAT signals are predominately involved in regulating the activity of numerous proteins involved in cell survival (Bcl-2, BAX, BAD) (116, 117, 126, 249, 294). In contrast to the well-defined role of JAK/STAT signaling, IL-7 activates only two branches of the MAPK pathway, SAPK/JNK and p38MAPK, but not the canonical MEK/ERK signaling pathway (13, 56, 259). Although IL-7 activates p38 and JNK their contribution to IL-7 induced proliferation and survival does not appear to be criticial (13, 20, 56, 228, 290).

The second major IL-7 induced pathway mediated by PI3K (Figure 5-2) has also been studied in depth and is canonically involved in cell proliferation through regulation of proteins involved in cell cycle checkpoints (12, 20, 126, 128, 152, 207). Upon IL-7 binding to the receptor, PI3K is recruited to the receptor, likely to Y449 as the docking site after JAK3 phosphorylation (116). PI3K phosphorylates the lipid substrate phosphatidylinositol-(4,5)-biphosphate (PIP2) to make phosphatidylinositol-(3,4,5)-triphosphate (PIP3) which serves as a lipid

anchor on the cell membrane to recruit additional downstream signaling intermediates such as AKT (13, 20, 88, 116, 255). AKT activation leads to the upregulation of genes involved in metabolism (GLUT-1 and CD71) (13, 128, 272), cell cycle control (p27kip1, GADD45A, p130, GSK3, cyclinD) (12, 13, 88, 152, 272), and cell survival (FOXO transcription factors, BAD, BCL-2, BIM) (235, 280, 298). The ubiquity of JAK/STAT and PI3K signaling in cytokine networks raises the possibility that cross-talk with other cytokines may be contributing to inhibiting IL-7 function. Thus, the goal of these studies was to assess the responsiveness of peripheral T cells in macaques with untreated SIV infection to determine whether these cells were able to respond correctly to IL-7 mediated homeostatic signals. The work presented here assesses the capacity of IL-7 to signal via the JAK/STAT pathway.

Results:

In-vivo Response to Homeostatic Signals is Impaired in SIV-infected Rhesus Macaques

In these studies we used both chronically infected macaques, animals SIV infected for more than 100 days and clinically stable, as well as end-stage macaques, SIV+ for more than 100 days that exhibited signs of sAIDS and required euthanasia. Viral loads in both cohorts ranged from 10^5-10^8 copies/mL. We did not observe any significant rsIL-7gly associated change in viral load, although end stage macaques had minor increases (less than 1 log variance) immediately prior to death 7-14 days post rsIL-7gly administration. End-stage macaques were additionally characterized by a basal Ki67 expression level less than 20% in CD4+ CM cells (chronically infected macaques >20%) and were more severely depleted of CCR5+ CD4+ cells than chronically infected animals. One possible explanation for the collapse in the CM compartment during chronic SIV infection is decreased responsiveness to homeostatic stimuli. To test this hypothesis, two doses of IL-7 were administered 7 days apart to: 1. Uninfected macaques; 2. Chronically SIV infected macaques; and, 3. SIV+ macaques exhibiting signs of end-stage disease.

Our hypothesis was that macaques with the most severe disease (end-stage) would be least responsive to rsIL-7gly administration in-vivo. Uninfected macaques demonstrated robust Ki67 induction in both the CM and naïve cell compartments

as expected (Figure 5-3). As we predicted, chronically SIV infected macaques exhibited significantly reduced Ki67 induction compared to uninfected macaques in both the CM and naïve compartments (Figure 5-3A). Surprisingly, macaques with end-stage infection showed a more robust Ki67 response than macaques with chronic infection (Figure 5-3). Additionally, the peak of the Ki67 response occurred earlier in the macaques with end-stage disease than in uninfected macaques though the increase in levels of Ki67 at the peak of each response was attenuated in the end-stage animals with uninfected ranging from a 15%-60% increase and end stage macaques range from 11-30% (Figure 5-3A). CD8+ T cells were more responsive in general than the CD4+ T cells in both uninfected and SIV infected macaques irrespective of disease state (Figure 5-3B). rsIL-7gly administration elicited significantly less Ki67 in both CD8+ naïve and CM subsets in chronically SIV infected macaques (red line) when compared to uninfected macaques (black line) (Figure 5-3B). The end-stage macaques (blue line) again showed a more robust Ki67 response when compared to the chronically infected macaques (red line) though the response was attenuated particularly in duration, when compared to the uninfected macaques (black line) (Figure 5-3B). As we observed in the CD4+ T cells the peak of the response occurred earlier in the end-stage animals after a single injection of rsIL-7gly (Figure 5-3).

To determine whether the reduction in the magnitude and duration of Ki67 expression impacts rsIL-7gly induced increases in T cell count the absolute number of CD4+ and CD8+ T cells was monitored during treatment. rsIL-7gly administration transiently increased the absolute number of T cells in the peripheral blood when given to uninfected macaques (black lines) from two (CM) (Figure 5-4B) to five (naïve) fold (Figure 5-4A). Counter to our hypothesis, rsIL-7gly also increased approximately two fold CD4+ CM (Figure 5-4B) and N (Figure 5-4A) cells beyond the end of the study period in chronically infected (red lines) animals. Surprisingly, despite eliciting early increases in Ki67 expression, rsIL-7gly transiently increased both the naïve (Figure 5-4A) and CM (Figure 5-4B) cells in end-stage macaques peaking between day 14 and 21. The data in Figure 5-4 suggests that in some cases (eg CD4+ CM) the chronically infected macaques increase T cell numbers slightly better than the uninfected macaques. However, the difference in outcome in terms of cell number may actually reflect the comparatively few CD4+ T cells in the SIV+ macaques in relation to the number of cells in an uninfected macaque. The comparatively small number of CD4+ T cells in the SIV infected macaques requires a smaller increase in the absolute number of T cells to double, for example, than relatively abundant CD4+ T cells in an uninfected animal making it easier to see a full fold change in a smaller cell population. This caveat potentially minimizes rsIL-7gly effects on T cell levels in uninfected macaques when directly compared to SIV+ macaques.

When we examined CD8+ T cells, naïve CD8+ T cells (Figure 5-4A) in particular, increased to a higher level than their CD4+ counterparts in uninfected macaques. CD8+ CM cells were the only subset in this cohort to remain elevated beyond the end of the study period (Figure 5-4B). Despite the rsIL-7gly induced expansion of CD4+ CM cells in some macaques, SIV infection, regardless of infection status, impaired expansion of naïve (Figure 5-4A) and CM (Figure 5-4B) CD8+ T cells. We also observed that this inhibition tended to be more severe as disease progressed particularly in the CD8+ compartments. As discussed above, using fold change as a measure of efficacy is susceptible to variation in population starting size between cohorts. However, CD8+ T cells are present in uninfected, SIV+ and end-stage macaques at similar levels circumventing the caveat discussed above. This suggests that in this case, the CD8+ T cell levels may be a better indicator of homeostatic disruption in the periphery than their CD4+ counterparts. The attenuation of the CD8+ T cell response in the SIV+ animals indicates that the IL-7 non-responsiveness is due to the altered immune system (indirect) and not the direct SIV infection of the cells. In sum, these observations suggest that the response of SIV infected macaques to rsIL-7gly is substantially different from the response of uninfected macaques with regard to the ability of rsIL-7gly to elicit proliferation in peripheral T cells.

IL-7 response in SIV infected macaques is not diminished by reduced expression of CD127

To assess the integrity of rsIL-7gly signaling downstream of the receptor a cross sectional study of uninfected and chronically infected macaques at the Oregon National Primate Research Center was undertaken. To assess whether IL-7R expression was comparable to uninfected macaques during chronic SIV infection on CD4 T cells we determined the percentage of CD127+ cells by flow cytometry. There was no significant difference between uninfected and SIV+ macaques in the percentage of cells expressing CD127 in both the CD4+ (Figure 5-5A) and CD8+ (Figure 5-5B) CM and naïve compartments. These data suggest that the attenuated proliferative response seen in the peripheral T cells is due to a defect in intracellular response rather than to the down-modulation of IL-7 receptor expression.

IL-7 signaling through STAT5 is impaired during SIV infection

One of the two major pathways shared by homeostatic cytokines that use the common gamma chain in their receptor complex (e.g. IL-2, IL-7 and IL-15) is the JAK/STAT pathway [reviewed extensively by (116). To determine whether this signaling pathway is impaired in CD4+ cells during SIV infection we asked whether ex-vivo stimulation with rsIL-7gly activated STAT5. PBMCs were stimulated ex-vivo with rsIL-7gly (16ng/1e^6 cells) for 15 minutes and phosphorylated STAT5 (STAT5P) detected by flow cytometry. Following rsIL-

7gly stimulation, naïve CD4+ T cells (CD28moderate, CD95low, CCR7moderate) from chronically infected macaques increased STAT5P+ cells significantly more than uninfected macaques (Figure 5-6A). By contrast, in these same animals, the percentage of STAT5P+ total memory CD4+ T cells (CD95+) tended to be lower than the levels attained by SIV- macaques (Figure 5-6A). The total memory compartment includes CM, TrM and EM subsets. However, when we refined the analysis to exclude CD127dim memory subsets (TrM ($CD28^{hi}$, $CD95^{hi}$, $CCR7^{dim}$) and EM ($CD28^{dim}$, $CD95^{hi}$, $CCR7^{dim}$)) and compared STAT5 activation in only CD4+ CM ($CD28^{hi}$, $CD95^{hi}$, $CCR7^{hi}$) cells we did observe significant attenuation in their response during chronic infection (Figure 5-6B). These results support our hypothesis that active SIV replication is associated with altered IL-7 signaling in peripheral CD4+ memory cells.

One possible source of the attenuation is inhibition of rsIL-7gly signaling by negative feedback inhibition resulting from high levels of endogenous gamma chain cytokines. We quantified plasma concentration of gamma chain cytokines associated with homeostasis as well as a cytokine associated with immune activation In T cells (IL-8) using Luminex technology. The combined plasma concentration of the major common gamma chain cytokines, IL-2, IL-7 and IL-15, was not significantly different in uninfected and infected macaques (Figure 5-7A). However, the median concentration of IL-7 in uninfected macaques (4.1

pg/mL) was significantly higher than the median concentration (2.0 pg/mL) in the SIV+ macaques. By contrast, the plasma concentration of IL-2, a cytokine critical for antigen specific T cell responses, was elevated in the SIV+ macaques (median concentration 14.8 pg/mL) compared to the uninfected macaques (median concentration 5.4 pg/mL; p=0.054) (Figure 5-7A). These results led us to assess endogenous level of an immune activation related cytokine, IL-8, which indeed was significantly higher (89.6 pg/mL) in the SIV+ macaques compared to uninfected macaques (24.1 pg/mL)(Figure 5-7B). These data suggest that the reduction in IL-7 induced STAT-5 phosphorylation in CD4+ CM T cells during chronic SIV infection is likely to be caused by aberrantly high levels of immune activation, and we did not uncover any evidence for a negative feedback inhibition from high endogenous levels of homeostatic cytokines.

Discussion:

One of the unresolved questions in the HIV field is why an aggressive acute infection takes years to manifest as overt AIDS. The rhesus macaque model has shown that one of the key correlates of disease progression during untreated SIV infection is depletion of CD4+ EM T cells at mucosal sites (208, 225). Recent work has suggested however, that the rate of disease progression is associated with the rate at which the CD4+ CM T-cell compartment looses its ability to replenish the EM compartment (208). IL-7 has emerged recently as a master

regulator of T-cell homeostasis and is of particular interest in these studies for its role in maintaining the CM T-cell compartment. These observations led us to question whether the homeostatic functions of IL-7 in particular were compromised during SIV infection. We hypothesized that this dysfunction would increase as the disease progressed from a chronic infection to simian AIDS.

Does a dysfunction exist in response to IL-7 during SIV infection?

To test whether IL-7 responsiveness in T cells was impaired during untreated SIV infection we administered rsIL-7gly to 14 untreated SIV+ rhesus macaques. These animals were subdivided into macaques during the chronic phase of infection (infected for more than 100 days and clinically stable in the absence of ART) and end-stage (infected for more than 100 days and requiring necropsy). We compared IL-7 function in these macaques to a cohort of age and sex matched uninfected macaques. In these macaques we observed that the peak and duration of the Ki67 response elicited in both CD4+ and CD8+ T cells was lower in SIV infected macaques than in uninfected macaques supporting our hypothesis that IL-7 function is altered during SIV infection (Figure 5-3). The rsIL-7gly response was equally attenuated in CD8+ T cells suggesting that the mechanism(s) contributing to IL-7 dysfunction are not unique to CD4+ T cells. In contrast to our expected results, chronically SIV infected macaques demonstrated increased CD4+ naïve and CM T cells in response to rsIL-7gly that persisted over the

follow up period, though the CD8+ T cells remained near baseline levels (Figure 5-4). This was true during chronic infection when Ki67 induction was attenuated in CD8+ T cells (Figure 5-3) and during end stage infection when rsIL-7gly administration elicited early, though attenuated, Ki67 expression in these cells (Figure 5-3).

One hypothesis to explain the divergence between the cohorts in terms of increasing CD4+ CM number and attenuated Ki67 response is that expansion and maintenance of the expanded population are dependent on the dual functions of IL-7 such that the initial expansion is dependent on induced proliferation and the maintenance of the newly expanded population on anti-apoptic signals. To broadly apply this line of reasoning: in an uninfected macaque increased proliferation in response to rsIL-7gly increases the number of peripheral T cells. These cells are then maintained via intact JAK/STAT signaling. However low basal levels of normal homeostatic proliferation leads to an eventual decay resulting in a transient increase when IL-7 is not driving accelerated proliferation. By contrast, chronically infected macaques have the highest basal levels of proliferation (> 20%) and exhibit only minor increases in %Ki67+ cells (~10%) in response to IL-7. The newly divided cells in these animals might potentially be maintained despite the aberrant reduction in JAK/STAT signaling by the high basal levels of proliferation driven by immune activation. In effect, there are now

double the cells with >20% of the population dividing thus slowing the decay in cell number as it returns to normal. Finally, to apply this line of reasoning to end-stage monkeys with low basal levels of proliferation; rsIL-7gly increases proliferation by approximately 20% in the peripheral T cells causing the initial expansion. However, JAK/STAT signaling is impaired reducing cell survival and the environment has already caused proliferative failure so the low level of basal proliferation is insufficient to maintain the newly expanded population size resulting in slight and transient increases in T cell number. In both SIV+ cohorts these increases in absolute cell number are in a relatively small population causing the fold change to appear larger than the expansion of a similar number of cells in a larger population.

Our experiments provide several convergent lines of data indicating that the mechanism impairing the IL-7 proliferative response is likely to be systemic. First, SIV infected macaques have an attenuated Ki67 response in both SIV target cells (CD4+ CCR5+ memory cells) and non-SIV target cells (CD4+ naïve and CD8+ T cells) indicating that the loss of IL-7 responsiveness is not the result of viral infection in an individual cell. Second, we observed only minimal changes in virus replication suggesting that viral killing is not accelerated in rsIL-7gly treated macaques. Finally, we found elevated plasma concentrations of both IL-2 and IL-8 during chronic infection (Figure 5-7). Our studies indicate that plasma

concentration of IL-7 in these macaques, as well as the total concentration of gamma chain using cytokines, is lower or no different than uninfected macaques (Figure 5-7). Thus, it seems unlikely that systemic lymphopenia is activating negative feedback loops, and perhaps more likely that cross-talk is occurring between cytokines associated with immune activation and the IL-7 signaling pathways to inhibit normal IL-7 function.

IL-7 Signaling:

Aberrant immune activation resulting from SIV infection may have several points of intersection with the IL-7 signaling axis. In these studies we monitored expression levels of CD127, the IL-7 receptor, and the ability of peripheral T cells to phosphorylate STAT5 in response to rsIL-7gly ex-vivo. Previous work has demonstrated that signaling in the IL-7/IL-7R axis is disrupted during HIV/SIV infection (1, 22, 23, 27, 28, 45, 87, 131, 132, 139, 172, 173, 183, 200, 211, 212, 216, 233, 242, 253, 291, 300). Both NHP and clinical studies have shown that over time, the percentage of cells expressing CD127 decreases in the CD4+ and CD8+ T-cell populations (22, 53, 54, 131, 162, 183, 211, 212, 233, 242). However, some of these studies fail to account for the proportional expansion of $CD127^{lo}$ EM cells (22, 183, 233, 242). An elegant study by Colle et al demonstrated that PBMCs from HIV+ patients with lower surface expression (MFI) of CD127 compared to uninfected donors were still able to upregulate

BCL-2 expression and proliferate (54). This study, and the work presented here, indicates that CD127 expression is sufficient to induce signaling. The next downstream stage of IL-7 signaling we assessed was STAT5 phosphorylation. In response to IL-7, the percentage of naïve cells activated is actually augmented during SIV infection as opposed to the reduction seen in total memory cells. One possible explanation is that new naïve cells are constantly emigrating to the periphery from the thymus and thus have not been exposed the aberrant cytokine levels in the periphery as long as memory cells. The attenuation in STAT5 activation observed in CD4+ CM cells supports our contention that IL-7 function is disrupted in these cells. The association between JAK/STAT signaling and apoptotic regulators also lends support to the idea that the inhibition of IL- signaling may affect cell survival as well as proliferation.

What distinguishes the end stage versus chronically infected animals?

One unresolved question in these studies is why end-stage macaques have an early burst of Ki67 expression in response to IL-7 that, though attenuated compared to uninfected macaques, is more robust than the chronically infected animals. One possibility is that the proliferative collapse sustained in the CM cells of macaques at end-stage (basal Ki67 expression <20%) creates a pool of resting cells potentially responsive to rsIL-7gly. Again speculatively, the collapse could stem from cross talk between the MEK/ERK and the PI3K pathway at the

checkpoint for G1 to S phase transition which is regulated by both cyclins and CDK complexes (9, 13, 20, 116, 295). During SIV/HIV infection, there are several possible sources of aberrantly high MEK/ERK signaling including antigen related crosslinking of the TCR (9, 199, 223, 224, 250, 254, 277) and/or aberrantly high levels of inflammatory cytokine expression (74, 295). MAPK signaling in response to opportunistic pathogens has also been shown to increase the level of inflammatory cytokines being produced suggesting yet another way aberrant activation of this pathway may occur (26, 282). In addition to generic effects from non-specific activation, HIV/SIV proteins have been implicated in inhibiting cell cycle progression directly via the MEK/ERK pathway including env (224, 250, 277) and nef (199, 205), as have opportunistic pathogens associated with AIDS (50, 120, 133, 222, 254, 264). MEK/ERK signaling at high levels is generally associated with increased expression of the cell cycle inhibitor p27kip1 (47, 214, 223, 255). However, with sufficient stimulation through JAK3, a MEK/ERK induced block can be overcome in peripheral T cells ex-vivo to initiate cell cycling (47, 250). The studies by Chen et al and Selliah et al would suggest a precedent for IL-7 mediated JAK3 signaling at high relative concentrations overcoming MEK/ERK inhibition in-vivo. To apply this line of reasoning to our studies, as the chronic stage of infection progresses to the end stage it is possible that macaques slowly develop a pool of cells that have a proliferative block due to aberrant MEK/ERK signaling which accounts for the

collapse of CM cell proliferation in the end stage macaques. IL-7 signaling via JAK3 at high concentrations (both from low numbers of T cells and administration of exogenous IL-7 creating a high relative concentration per cell) may then be sufficient to overcome the MEK/ERK mediated inhibition resulting in a detectable "wave" of Ki67 expression as we see in figure 5-3.

Implication of this research in the broader pathogenesis field:

The data presented here indicates that during pathogenic SIV infection, the homeostatic response to IL-7 is impaired both in its ability to increase proliferation in peripheral T cells and its ability to activate STAT5. Attenuation of major endogenous homeostatic signal in an already lymphopenic system may contribute to accelerating the rate at which self-renewing T cells decline ultimately accelerating the progression to AIDS. The aberrations we demonstrate in this system suggest it is unlikely that administering IL-7 as an immunotherapeutic in the absence of effective HAART treatment would succeed. Moreover, the potential consequences of non-specifically expanding immune cells in an activated environment are not well understood. Further work in the macaque model will aid in developing our understanding of the IL-7/IL-7R axis and the consequences of this deregulation.

FIGURE 5-1: Simplified signaling in the JAK/STAT pathway downstream of the IL-7R

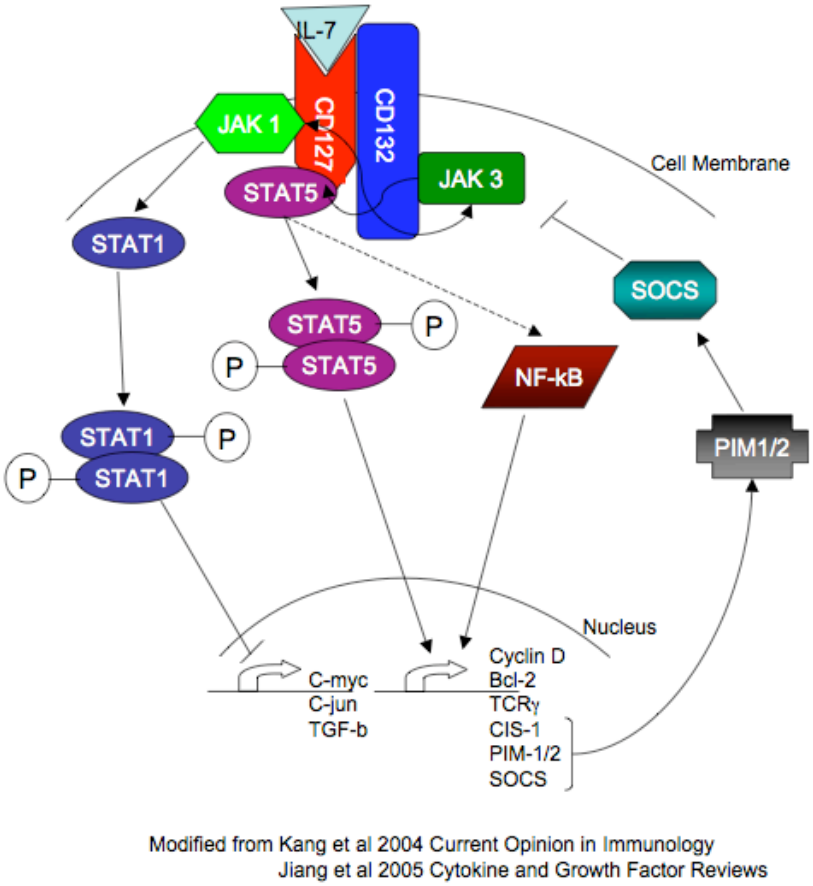
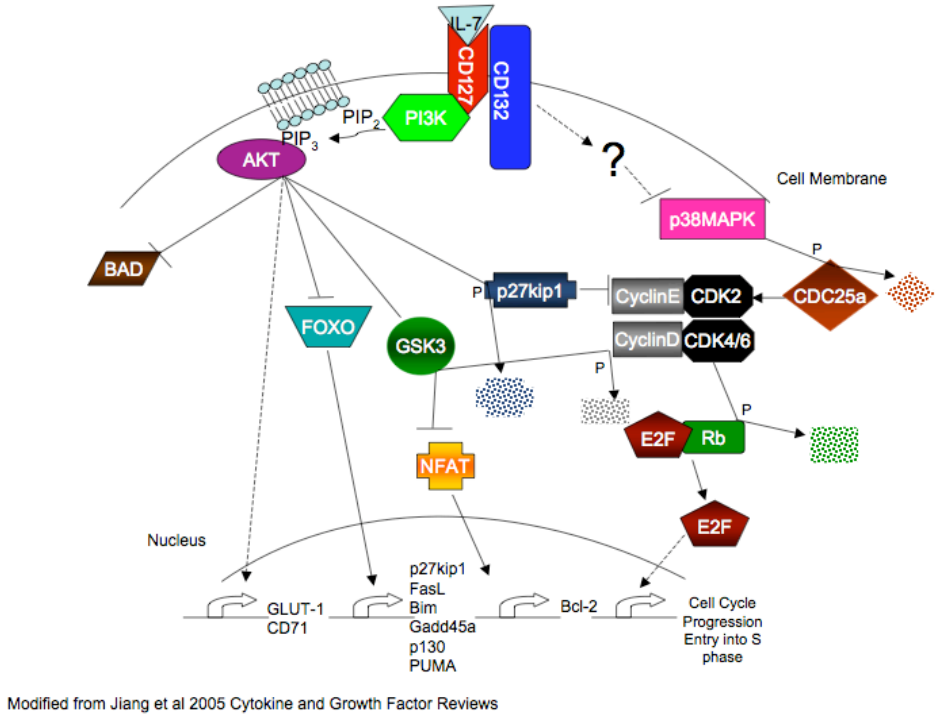


FIGURE 5-1: JAK/STAT Signaling Overview. Upon IL-7 binding to the IL-7R the IL-7 specific alpha chain dimerizes with the common gamma chain. This leads to an autophosphorylation event within the receptor complex that creates docking sites for JAK1 and JAK3. JAK proteins in turn activate STAT proteins, STAT5 being the predominate STAT involved in IL-7 signaling, by phosphorylating specific tyrosine residues on the STAT. After phosphorylation, STAT proteins form dimers or larger complexes and translocate to the nucleus where they activate gene transcription.

Figure 5-2: PI3K Mediated Signaling Downstream of the IL-7R



Modified from Jiang et al 2005 Cytokine and Growth Factor Reviews

Figure 5-2: Simplified IL-7 signaling through the PI3K pathway. Upon IL-7 binding to the receptor, PI3K is recruited to the receptor, likely to Y449 as the docking site after JAK3 phosphorylation. PI3K phosphorylates the lipid substrate phosphatidylinositol-(4,5)-biphosphate (PIP2) to make phosphatidylinositol-(3,4,5)-triphosphate (PIP3) which recruits AKT through its PH domain. AKT activation has multiple downstream effects: 1) Phosphorylation of BAD causing its retention in the cytosol, 2) upregulation of metabolic genes, 3) phosphorylation of FOXO transcription factors particularly FOXO1 and FOXO3 leading to their cytosolic retention inhibiting their ability to transcribe target genes; 4) phosphorylation of GSK3 which both blocks NFAT and phosphorylates Cyclin D leading to activation of CDK2; and 5) phosphorylation of p27kip1 leading to its degradation relieving inhibition of CDK proteins. Relieving inhibition by p27kip1 and cyclins allows active CDKs to phosphorylate Rb causing its degradation and release of active E2F that drives S phase entry in the cell cycle.

Figure 5-3: Ki67 Expression Dynamics Following rsIL-7gly Administration

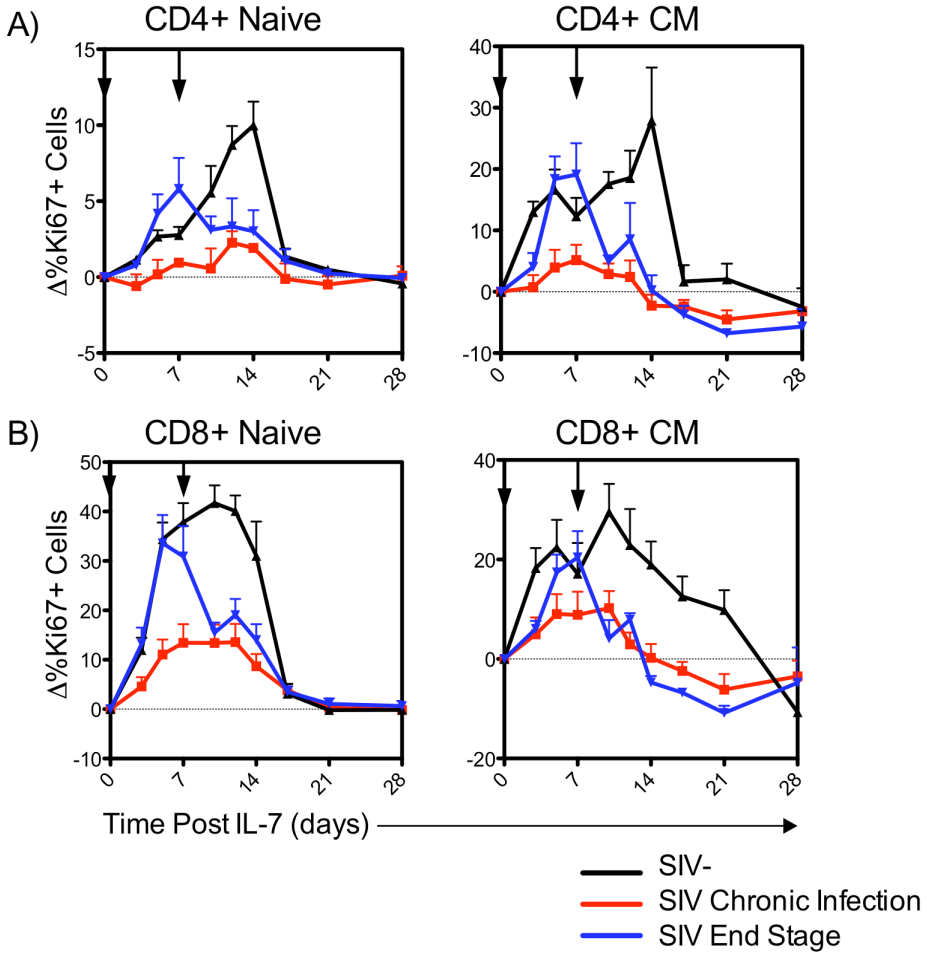


Figure 5-3: Dynamics of Ki67 expression following rsIL-7gly administration in macaques with untreated SIV infection. Ki67 expression was monitored in T-cell subsets as defined in materials and methods using flow cytometry for 28 days following rsIL-7gly administration indicated as [↓] at day 0 and day 7. Basal Ki67 expression (the percent of proliferating cells at day 0) was normalized for all cohorts by averaging three timepoints pre-rsIL-7gly administration. Uninfected controls (black N=8); Chronically infected macaques (red N=10); End-stage infection (blue N=4). A) Change in Ki67 expression over time for CD4+ T-cell subsets B) Change in Ki67 expression over time in CD8+ T-cell subsets

Figure 5-4: Effect of rsIL-7gly on Absolute T cell Number

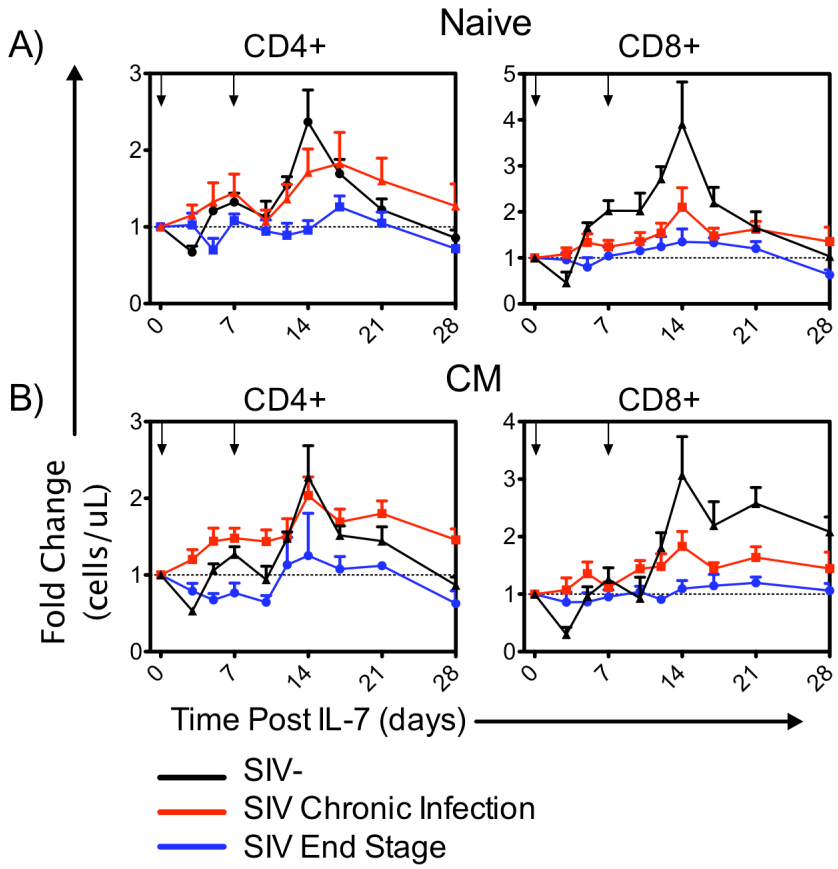


Figure 5-4: Change in absolute T-cell levels following rsIL-7gly administration during untreated SIV infection. The absolute T-cell levels following rsIL-7gly administration at day 0 and day 7, indicated by [↓] are shown for all three cohorts. The absolute counts are shown as the mean fold change (+/- SEM) from levels pre-rsIL-7gly administration (average of three timepoints) that is set at 1. Uninfected controls (black N=8), chronically infected macaques (red N=10), macaques with end-stage SIV infection (blue N=4). (A) Naïve T cells (B) CM T cells.

Figure 5-5: IL-7R Expression on Peripheral T cells during SIV Infection

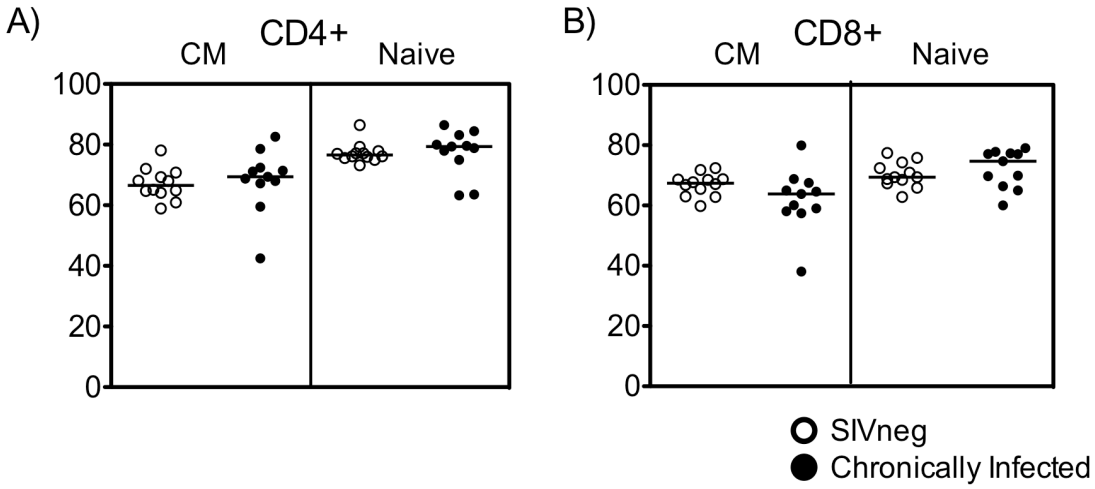


Figure 5-5: IL-7R (CD127) expression on peripheral T-cell subsets during chronic SIV infection prior to rsIL-7gly administration. IL-7R expression was assessed using flow cytometry prior to rsIL-7gly administration to determine whether IL-7R expression was impaired in chronically infected macaques. Our results indicate that IL-7R expression in the CM and naïve subsets is comparable in uninfected control macaques (open symbol) and chronically SIV infected macaques (closed symbol). No significant differences in receptor expression were identified using the Mann-Whitney T-test.

Figure 5-6: STAT5 Activation in Response to rsIL-7 Stimulation Ex-vivo

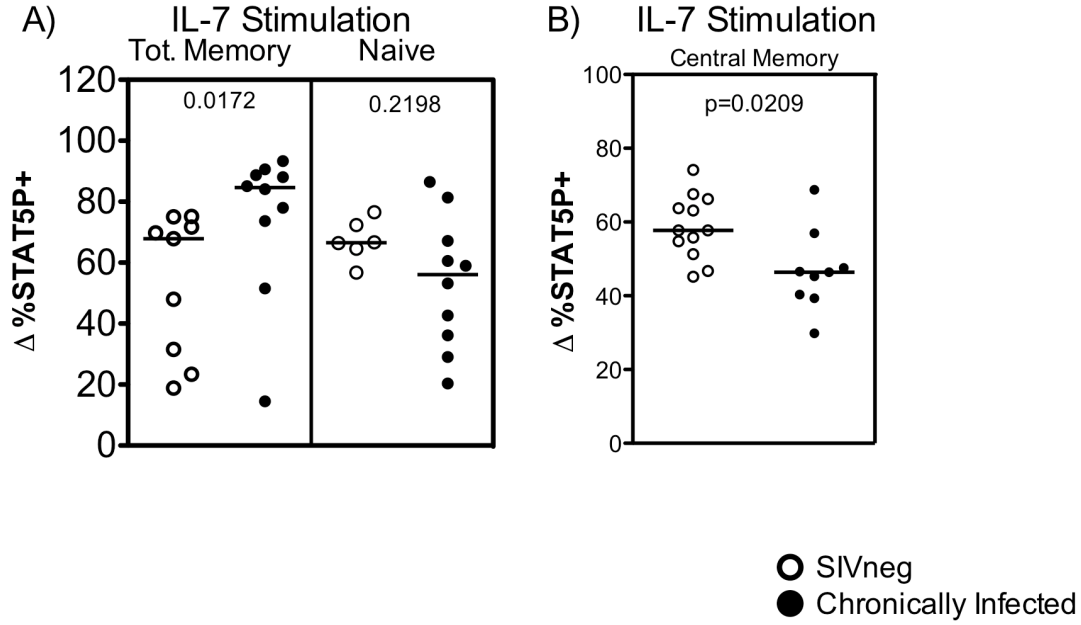


Figure 5-6: Phosphorylation of STAT5 in response to both IL-7 and IL-15 stimulation ex-vivo is impaired in chronically SIV infected macaques. Total PBMCs were stimulated with either IL-7 for 15 minutes ex-vivo and phosflow cytometry was used to identify STAT5P in T-cell subsets. Significant differences between uninfected macaques (open symbols) and chronically SIV infected macaques (closed symbols) were determined using the Mann-Whitney T-test and where appropriate exact p values are shown. A) Change in percent of STAT5P+ naïve or total memory CD4+ T cells following IL-7 stimulation. B) Change in STAT5P+ CD4+ CM T cells following IL-7 stimulation.

Figure 5-7: Plasma Concentrations of Common Gamma Chain using Cytokines during SIV Infection

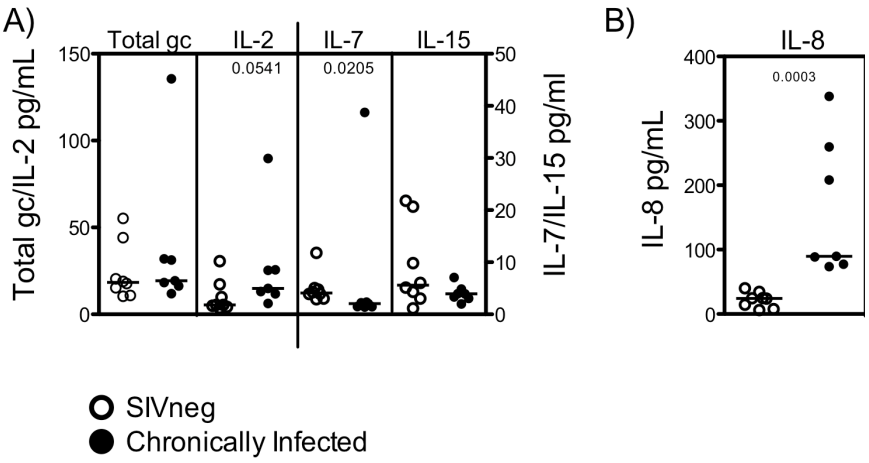


Figure 5-7: Plasma concentration of endogenous cytokines during SIV infection prior to rsIL-7gly Administration. Multiplex luminex technology was used to determine the concentration of endogenous homeostatic (IL-2, IL-7 and IL-15) and immune activating (IL-8) cytokines. The concentrations of each in uninfected and SIV+ macaques prior to rsIL-7gly administration are shown. The concentration of total gamma chain using cytokines was determined by adding the concentration of IL-2, IL-7 and IL-15. Significant differences between the cohorts were determined using the Mann-Whitney T-test and where appropriate exact p values are indicated. Open symbols- uninfected controls; close symbols- SIV+ macaques.

CHAPTER SIX

DISCUSSION AND FUTURE AIMS

Overarching Aim of the Thesis:

During an HIV infection, the CD4+ T-cell compartment is depleted directly by the virus and indirectly as a byproduct of aberrantly high levels of immune activation. Current antiretroviral strategies target susceptible stages of the virus lifecycle (entry, reverse transcription, integration and protease activity) for inhibition and are successful in most patients. Immmunotherapeutics are an emergent class of potential interventions to be used as a compliment to current HAART. This concept led to the proposal that homeostatic cytokines, naturally responsible for balancing and maintaining T-cell number, could benefit patients. IL-7, IL-2 and IL-15 have been proposed as the leading candidates for reconstituting the T-cell compartment in HIV+ patients (20, 148, 161, 246). IL-7 has however emerged as the strongest candidate for its role in promoting and maintaining the naïve and CM T-cell subsets (145, 236, 286).

The overarching goal of this dissertation was to better characterize IL-7 as an immunotherapeutic during SIV infection in the rhesus macaque model. The hypothesized benefits of using IL-7 as an immunotherapeutic can be categorized

in two ways: 1) direct benefit to the T-cells and 2) ancillary effects improving overall immune health. We show here that IL-7 is able to restore the CD4+ T-cell subsets killed by the virus (Chapter 3) most likely through a combination of increasing proliferation and survival. We also show that it is possible IL-7 may have ancillary benefits for naïve B cells (Chapter 4). In the course of these studies we also developed an interest in understanding how responsiveness to IL-7 is impaired by active or sub-optimally suppressed virus replication. In addition, to better understand the mechanisms by which SIV infection attenuates responsiveness to IL-7 we undertook the second series of experiments presented in chapter 5.

Developing Interleukin-7 as a Candidate Immunotherapeutic in the NHP model:

The primary study presented here was undertaken to ascertain the optimal dosing regimen for IL-7 administration in the macaque model. The earliest studies administering IL-7 to NHPs were published in 2003 (92, 204). These studies administered recombinant human IL-7 (rhIL-7) produced in E.coli, which likely increased the immunogenicity of the IL-7, though neither study reported neutralizing antibody (92, 204). Neither Fry et al nor Nugeyre et al extensively identified T-cell subsets on the basis of phenotypic markers to assess the impact of rhIL-7 administration on peripheral T-cell subsets (92, 204). However, both of these early studies are proof-of-principle that administration of IL-7 increases T-

cell number (92, 204). Subsequent work in the primate model confirmed the results presented by Fry et al (92) and Nugeyre et al (204) using recombinant simian IL-7 (rsIL-7) in both macaque (24, 65, 111, 184) and baboons (160). The rsIL-7 used was generated in E.coli and non-glycosylated leading to reported immunogenicity and, in some cases, the production of neutralizing antibodies (24, 65, 184). The dosing regimens in these early studies were 80-100ug/kg/d (92, 160, 204) or regimens of 100ug/kg/d every other day (24, 65, 184) (Table 6-1). Only one study prior to our work used doses less than 80ug/kg/d (184) or used intervals greater than 48 hours between doses (65). The most recent primate study used the same glycosylated recombinant simian IL-7 (rsIL-7gly) (Cytheris) that we used in our studies but at a single high dose (25). We established our initial dosing interval at 7 days between rsIL-7gly injections based on a 2007 NHP study published by Dereuddre-Bosquet et al (65). In this study the authors tested high dose (100ug/kg/d) non-glycosylated rmIL-7 with 2, 4 or 7-day intervals between doses. The highest total levels of Ki67 and Bcl-2 expression over time were elicited when rmIL-7 was administered at 2-day intervals (65). However, Ki67 expression increased at approximately 7-day intervals even if rmIL-7 was administered in the interim (65). This suggested to us that the cells were not responding to rmIL-7 by proliferating more than once every 7 days. Based on this study and the extended half-life of rsIL-7gly when compared to earlier generations (i.e. rmIL-7) in-vivo (data from Cytheris not shown) we chose

to administer rsIL-7gly at 7-day intervals within each cluster. Our work is unique in using low dose administration (30ug/kg/wk) and "wash-out" periods between doses. The studies presented here are the first systematic attempt to optimize recombinant IL-7 administration in the macaque model by testing different intervals between exposures.

Efficacy of rsIL-7gly Treatment in Reconstituting the T-cell compartment in ART controlled SIV infection:

Chapter 3 outlines the response of peripheral T cells to administration of glycosylated recombinant macaque IL-7 (rsIL-7gly) in the rhesus macaque model. In our first short term study we demonstrated that administration of the cytokine to uninfected macaques elicited both increased proliferation and absolute cell number (Figure 3-1 and 3-2). The results demonstrate that at low doses (30ug/kg/d) and at 7-day intervals rsIL-7gly retains sufficient efficacy to justify our choice of dosing within the cluster. In our studies, a single dose of rsIL-7gly elicited peak Ki67 expression approximately 5-7 days after administration and returning to basal levels by day 14 (Figure 3-1, 3-3 and 3-4). However, by clustering rsIL-7gly we were able to extend the length of time cells were Ki67+ suggesting that we were able to increase the duration of the proliferative response so that it was at peak levels from day 7-21 and returned to baseline by day 28 (Figure 3-1 and 3-5). The clustered dosing also elicited the most sustainable long-term increases in absolute T cell count (Figure 3-6). Based on the studies in

chapter 3 we concluded that in this model, the synergistic effects of clustered rsIL-7gly doses was the best regimen to elicit long term increases in naïve and CM T cells.

rsIL-7gly Administration Effects B cells as well T cells

SIV, like HIV, is a disease characterized by dysfunction in multiple immune cell subsets. To determine whether rsIL-7gly may be beneficial for overall immune health in we assessed the impact of treatment on CD3+CD4-CD8- T cells which may have T-helper or regulatory properties (Chapter 4). Recent studies assessing a CD3+ T cell subset in African green monkeys (a natural host of SIV) that does not express CD4 and only the CD8-alpha subunit have determined that T cells without CD4 could potentially have T-helper cell functionality (17). Other studies in the Sodora laboratory have identified a population of CD3+CD4-CD8- T cells in a different SIV natural host species, sooty mangabeys, that are resistant to SIV infection and display some characteristics of helper T cells. In addition, studies from mouse models have determined that CD3+CD4-CD8- T cells could also have T-regulatory or immunosuppressive properties (48, 86, 276) as well as anti-tumor properties (299). We hypothesized that increasing the population of this T cell subset that might have helper T cell function but would be predicted to be resistant to SIV/HIV infection would be beneficial to the SIV/HIV infected immune system. However, our data indicates that rsIL-7gly administration had

only a minimal effect on either proliferation or cell number for CD3+/CD4-/CD8- T cell population (Figure 4-3).

A second immune cell subset assessed in chapter 4 for their response to rsIL-7gly administration was B cells. These data demonstrate that rsIL-7gly was able to increase the absolute number of naïve B cells approximately 2-fold in SIV+ ART treated macaques (Figure 4-6). We did not observe an increase in memory B cell levels (Figure 4-6). As we observed in chapter 3 for T cells, expansion of naïve B cell numbers was attained in the absence of robust Ki67 induction (Figure 4-4). This suggests that rsIL-7gly is acting in this model to increase production of naïve B cells in the bone marrow. Definitive studies undertaken to assess directly the impact of rsIL-7gly on B cell precursor populations in the bone marrow are necessary to address this hypothesis. In addition, a sampling protocol at higher frequency to assess whether the proliferation in naïve B cells occurs at timepoints not sampled in this study should be conducted to determine whether the peripheral population is proliferating and not being detected in these studies. Our studies indicate that rsIL-7gly is able to expand naïve B cells which could possibly increase resistance to novel opportunistic infections and may suggest a broader impact for rsIL-7gly than originally anticipated, beyond only impacting T cells.

Results from Pre-Clinical NHP studies and Clinical Trials are Highly Convergent:

Our findings with regard to the spacing and efficacy of rsIL-7gly administration in the macaque model are highly convergent with recently published human trials. In lymphopenic cancer patients two groups have demonstrated that 30ug/kg/d of rhIL-7 effectively increased CD4+ and CD8+ T-cell levels though not circulating B cells or neutrophils (237, 266). The key commonality of these studies is that IL-7 administration is clustered over a 2-3 week period and the benefits of rhIL-7 administration (increased lymphocytes) are observed for a period of time after rhIL-7 administration is stopped. In addition, Sportes et al (266) found that Ki67 expression increased transiently over a 14-day period despite multiple rhIL-7 doses. However, in this study the anti-apoptotic protein BCL-2 increased continuously over the course of rhIL-7 dosing (266), supporting our hypothesis that rsIL-7gly administration in the macaque model is increasing absolute T cells via survival as well as proliferation.

Two clinical studies more closely analogous to our pre-clinical macaque studies have been conducted in HIV+ patients receiving stable HAART in the last year. Levy et al administered 8 doses of rhIL-7 over 16 days (149) while Sereti et al (251) administered a single rhIL-7 dose. The Levy group observed transiently increased proliferation and elevated lymphocyte counts over 48 weeks post rhIL-7 administration in the naïve and memory T-cell compartments (149). By contrast,

the single injection administered by Sereti et al (251) elicited transient increases in Ki67 expression and minimally increased absolute T cells over the course of 28 days. We expand on the results presented above in both clinical (149, 237, 251, 266) and pre-clincal studies (24, 25, 65, 92, 111, 184, 204) by demonstrating that rsIL-7gly administration increases the half-life of T cells in the peripheral blood. In addition to recent work from Beq et al (25) demonstrating rsIL-7gly may reconstitute mucosal T cell compartments. Our results broaden the potential impacts of IL-7 immunotherapy.

Contribution of Endogenous IL-7 to HIV Pathogenesis:

The studies we present here administer rsIL-7gly in the context of an ART controlled SIV infection and suggest the use of rsIL-7gly as an immunotherapeutic only in that context. During the initial phase of our studies presented in chapters 3 and 4, two macaques (RM2-12 and RM2-13) failed to suppress viral replication on ART and were not included in the analyses presented. One of these macaques did however receive rsIL-7gly and failed to respond to the cytokine by increasing either Ki67 expression or absolute T cells levels (data not shown) providing anecdotal evidence that active infection may attenuate an IL-7 response. These observations led us to question how an untreated SIV infection intersects with IL-7 function.

Previous work has suggested interplay between IL-7 and HIV/SIV viral replication that may affect disease outcome. In-vitro (247) and patient studies (154), suggest that one danger of elevated IL-7 levels is an increase in CXCR4 tropic viruses that correlate with poor patient prognosis (154, 156). In-vitro studies conducted with CXCR4 tropic (71, 248) and primary HIV isolates (260) indicate that IL-7 administration increased HIV replication without inducing either markers of activation or a pseudo-memory phenotype in resting T cells. These studies indicate that elevated IL-7 levels in an untreated infection may be associated with increased viral replication particularly the more pathogenic CXCR4 tropic infection. Studies published in our laboratory previously suggest that during late phase chronic infection rhesus macaques infected with SIVmac239 the levels of endogenous plasma IL-7 increase suddenly and dramatically, but that there is no corresponding increase in either T-cell proliferation or levels (191). The results from the NHP model are again highly convergent with cross sectional data from HIV+ patients which indicates that plasma IL-7 levels increase as patients become more lymphopenic (84).

Does SIV infection impair Responsiveness of Peripheral T cells to IL-7?

We conducted the studies presented in chapter 5 to better characterize the effect of SIV infection on the in-vivo function of IL-7. These studies were conducted in uninfected, chronically SIV infected macaques and macaques infected with end-

stage SIV to gauge the response of peripheral T cells to homeostatic cytokines across a spectrum of SIV infection. The SIV+ cohorts had overlapping viremia and numbers of absolute memory T cells, however, the animals at the end stage of infection had a uniformly lower percentage of cells cycling prior to rsIL-7gly administration. Both SIV+ cohorts showed distinct deviations from the uninfected macaques in response to IL-7 treatment suggesting that SIV infection does impair endogenous IL-7 function.

Is intracellular signaling in response to rsIL-7gly impaired during SIV infection?

Numerous studies have documented the downregulation of IL-7R on peripheral T cells in the periphery possibly leading to fewer cells able to bind rsIL-7gly upon administration (22, 53, 54, 131, 162, 183, 211, 212, 230, 233, 242). In the work presented here by contrast, we did not detect a significant difference in the level of IL-7R expression on peripheral T-cell subsets from uninfected or SIV infected macaques (Figure 5-5). One possible explanation of this discrepancy is that the majority of the previous studies fail to account for the proportional expansion of EM cells which are CD127dim by flow cytometry in the general memory population (22, 183, 233, 242). In addition, Colle et al (54) demonstrated that similar levels of IL-7R are expressed on peripheral T cells from viremic patients (no HAART) and HAART treated patients with CD4+ levels below 250cells/uL blood. In these patients, following IL-7 stimulation ex-vivo, peripheral T cells

from HIV- control patients and the HAART treated patients with reduced CD127 expression upregulated Bcl-2 levels to similar levels, but Bcl-2 upregulation was attenuated in cells from the viremic patients (54). These convergent lines of evidence suggest that even if receptor expression varies between individuals viremia itself rather than the level of IL-7R expression causes the aberration in the intracellular response to IL-7.

Active SIV infection alters signaling via the JAK/STAT pathway:

Reports that HIV infection alter intracellular JAK/STAT signaling are numerous, inconsistent and include aberrantly high activity of multiple STAT proteins (1, 45, 132, 173), a reduction in the amount of wild type STAT protein present in cells (216) and inhibition of STAT5 signaling (139, 291). One group has consistently reported the presence of a dominant negative STAT5 mutant with the C-terminal truncated in peripheral T cells from HIV+ patients though these claims remain controversial (29-31, 57). One source of variability in these systems is that HIV strains have divergent impacts on JAK/STAT signaling due to differences in receptor cross linking by the viral envelope protein (139). Thus, to determine whether JAK/STAT signaling in our system was compromised we compared STAT5 phosphorylation in response to rsIL-7gly stimulation ex-vivo in an uninfected and chronically SIVmac239 infected macaques from ONPRC. In our studies we detected a reduction in STAT5 activation only in the CD4+ CM

compartment (Figure 5-6), suggesting that SIV infection does in some way alter signaling downstream of the IL-7R and that the modulation is most likely dependent on the stage of T-cell differentiation. Many of the previous results published have been presented as STAT activation within entire PBMC cultures (not T-cell subsets) (1, 139, 216, 291) or in cell culture lines (45, 132, 261). Thus our results suggest that another possible source of confusion in elucidating the effect of HIV/SIV infection on JAK/STAT signaling is both the type of cell line used and the representation of each stage of T cell differentiation in the PBMC culture used for the assays.

Surprisingly, in contrast to our previous work (191) our studies suggest that in a cross sectional analysis of chronically infected macaques, the endogenous plasma IL-7 levels is significantly lower than in uninfected controls. The results presented here do have precedent in human trials (200) though the factors contributing to these different results have not yet been identified. We propose that aberrantly high systemic immune activation is the most likely explanation that accounts for loss of rsIL-7gly in non-SIV target cell types and inhibition of IL-7 signaling in CD4+ memory T cells. In summary, in chapter 5 we show that SIV infection leads to an attenuated proliferative response to rsIL-7gly that in addition to compromised JAK/STAT signaling may contribute to the collapse in the CD4+ T cell compartment.

Final Summary:

The sum of the work presented in this thesis leads to two interlinking working hypotheses. We propose here two conflicting ideas: 1) SIV infection deregulates the IL-7 signaling axis leading to impaired function and 2) IL-7 is a promising immunotherapeutic that can be used to recover the T cell compartment. In conclusion I would like to present two working hypotheses demonstrating how in actuality these two observations are congruent and depend of the host background to determine IL-7 efficacy. The first working hypothesis (Figure 6-1) addresses the role of endogenous IL-7 as the infection progresses. Based on the work presented in Chapter 5 I would suggest that the proliferative response to IL-7 is not impaired during SIV infection, rather the loss of JAK/STAT signaling leads to impaired survival signals. Thus, both uninfected macaques and SIV+ macaques that have undergone homeostatic failure (end-stage) have detectable bursts of proliferation following exposure to exogenous IL-7. The discrepancy in pro-survival signaling however, fails to maintain the recently expanded population in the SIV+ macaques. The disruption in the JAK/STAT pathway can be hypothesized to stem from interplay between viral proteins (shown anecdotally by macaques with incomplete viral suppression on ART) and innate immune activation. However, I would propose that ART shifts the host environment from one containing high levels of viral proteins and highly activated to one more like

"normal" macaques (Figure 6-2). Thus, in SIV+ macaques the high background levels of proliferation (discussed in chapter 5) prevent any detectable increase in the percentage of Ki67+ cells when IL-7 is administered. Effective ART reduces the level of immune activation and helps restore normal levels of T cell turnover resulting in lower proliferation prior to the initiation of IL-7 treatment and a detectable burst in proliferation (Chapter 3). In the background of high levels of immune activation JAK/STAT signaling is likely impaired (Chapter 5) preventing survival and ultimately expansion of cell number in the absence of ART. With effective ART and lower immune activation the cell number and survival increase suggesting that the anti-apoptotic function of IL-7 has been restored (chapter 3). The NHP models discussed in this dissertation will continue to be necessary steps to demonstrating the validity of the hypotheses presented in this dissertation.

FUTURE AIM: Can IL-7 be used to augment immune function in HIV+ patients during co-infections and vaccination?:

One potential benefit of using of IL-7 as an immunotherapeutic may be augmenting the generation and maintenance of immunological memory. This could help elicit adaptive immune responses to a variety of co-infections associated with HIV morbidity including malaria (175), human papillomavirus (267), influenza (81) and tuberculosis (46). IL-7 acts on naïve T cells to increase the number of potentially antigen specific cells by increasing thymopoiesis (195), increasing the breadth of the naïve cell repertoire (266) and upregulating Bcl-2

expression (275). However, the ability of IL-7 to expand antigen specific CD4+ (147, 271) and CD8+ T cells (93, 171, 194, 270) during an antigen response is more likely to provide benefits for long-term immunological health. IL-7 has been shown to expand CD8+ EM T-cell number and function (93, 94, 270) in part by priming peripheral T cells to respond to TCR signals (94, 171). By lowering the threshold for T-cell activation IL-7 has been shown to augment the immunogenicity of subdominant epitopes within an antigen thus eliciting a broader EM cell response that persists in the CM cells generated (171). In the context of IL-7 as an immunotherapeutic, successfully using IL-7 to treat HIV infection may be able to expand the T cell repertoire as well as number. If IL-7 is successful as an adjuvant the patient population likely to benefit from optimizing administration increases to include patients that are immunosuppressed for multiple reasons such as genetic disorders, cancer treatment or organ transplant. A recent study indicated that the timing of IL-7 administration is critical to this function demonstrating that for optimal generation of long-lived memory exogenous IL-7 needs to be administered during the contraction rather than the expansion phase of an immune response (194). It has also been suggested that EM cells retaining expression of the IL-7R become long-lived CM cells (10, 130, 281).

The NHP model is a critical test site for determining the optimal dosing strategy for IL-7 to elicit long-term immunological memory to novel antigens in this scenario. IL-7 has been tested as a vaccine adjuvant in anti tumor models (55, 127, 150) though not in the primate system. To determine whether IL-7 had the potential to be used as vaccine adjuvant during immunocompromised states it would be necessary to first assess its potential in an immunocompetent host. To test this hypothesis I would administer a sample vaccination (e.g. H1N1 flu vaccine) to macaques subsequent to, concurrent with and prior to rsIL-7gly administration (Figure 6-3). To monitor the efficacy of IL-7 as an adjuvant would require in-vivo monitoring of labeled cells, particularly at mucosal sites where challenge exposures are likely, monitoring of T cell phenotype to determine whether long-term memory cells are generated, ex-vivo assessment of antigen specific responses using flow cytometry to monitor the strength of the response and finally assessment of B cell antibody production and maturation. The second phase of this type of experiment would be to challenge macaques vaccinated with and without IL-7 treatment with homologous pathogens (e.g. Swine flu) and heterologous challenges (e.g. 1918 Influenza) to determine whether the immunity generated in the presence of IL-7 was more or less protective. In this phase it would also be important to monitor the breadth of the immune response to determine whether sub-dominant epitopes in the vaccine were eliciting protection.

The final experimental sequence would be to conduct vaccinations at the optimal IL-7/pathogen interval in SIV infected macaques monitoring the same parameters.

Conclusion:

In conclusion, IL-7 as demonstrated the potential in the rhesus macaque model to be a broad acting therapeutic able to restore the depleted CD4+ T cell compartment. If this work translates as expected into patients, IL-7 as the potential to increase the quality of life and hopefully prolong the life of patients with a discordant response to HAART. The NHP model has been and will continue to be a valuable tool in developing and testing the novel uses of IL-7 in a therapeutic context.

TABLE 6-1: rIL-7 administration regimens used in previous studies

Citation	rIL-7 (ug/kg)	Dose interval	Duration of dosing	Supplier
Lu	80	Daily	8 weeks	
Nugeyre	40	2x daily	21 days	
Fry	100	Daily	9 days	
Beq 2006	100	Every other Day	26 days	
Moniuszko	100	Daily	15 days	
	100	Every other day	15 days	
	10	Every other day	15 days	
Dereuddre-Bosquet	100	Daily		Cytheris
	100	4-day		
	100	7-day		
Beq 2009	100	Once	n/a	Cytheris

TABLE6-1: This table shows the previously tested dosing intervals for rIL-7 administration in NHP models.

Figure 6-1: Working Hypothesis Addressing the Function of rsIL-7gly during SIV Infection

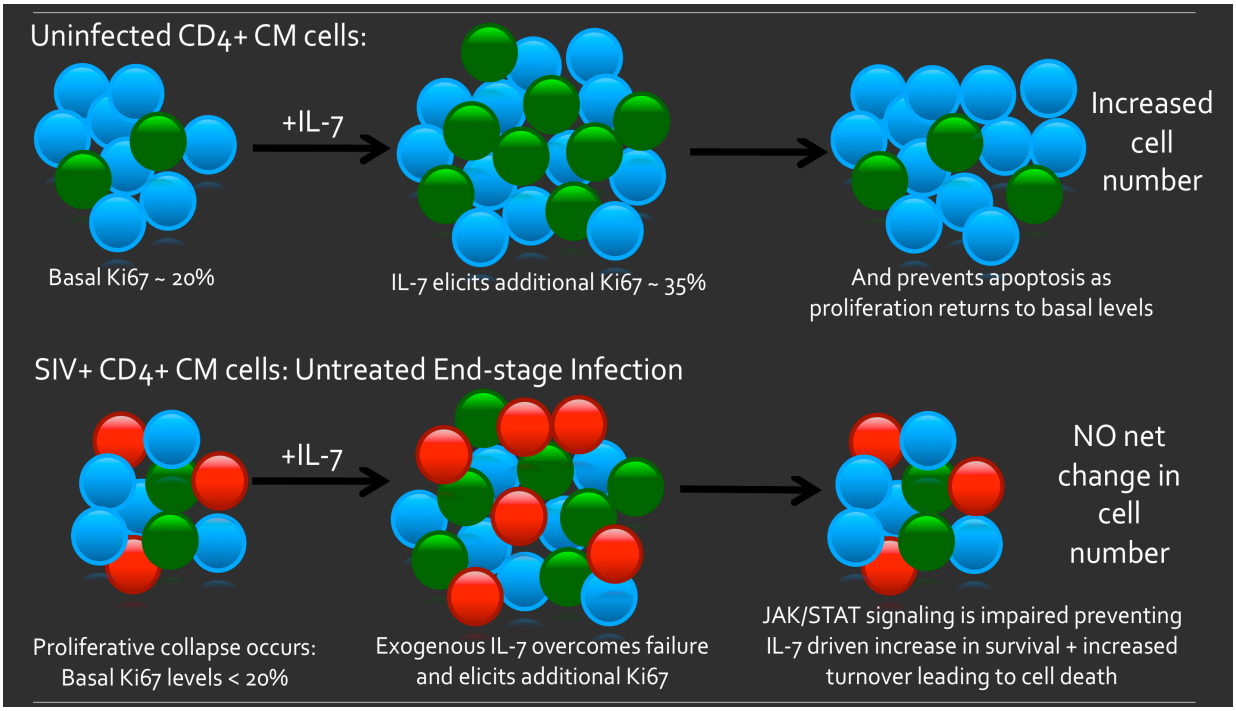


Figure 6-1: Model showing how rsIL-7gly can have differing outcomes in uninfected and SIV infected macaques during the end stage of infection with similar Ki67 expression profiles. Undividing resting T cells are shown in blue, Ki67+ cells are shown in green and SIV+ cells are shown in red.

Figure 6-2: Working Hypothesis Addressing why Effective ART is Crucial to Successful rsIL-7gly Therapy

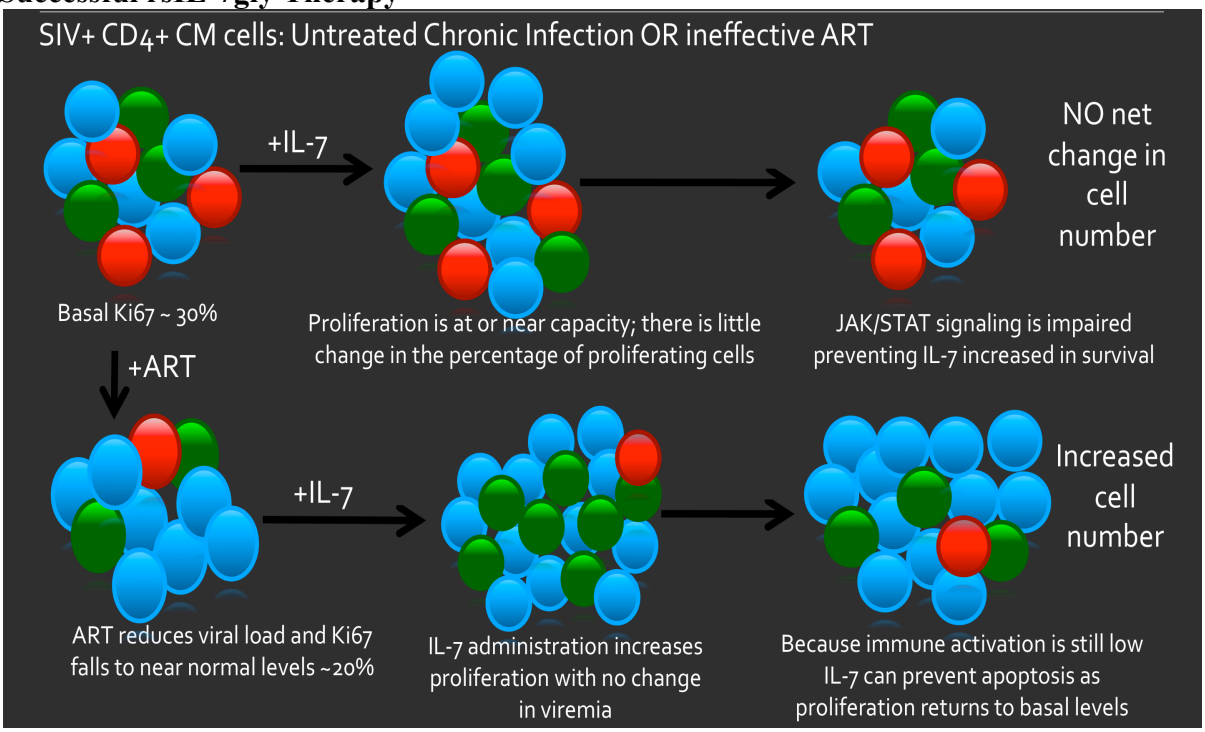
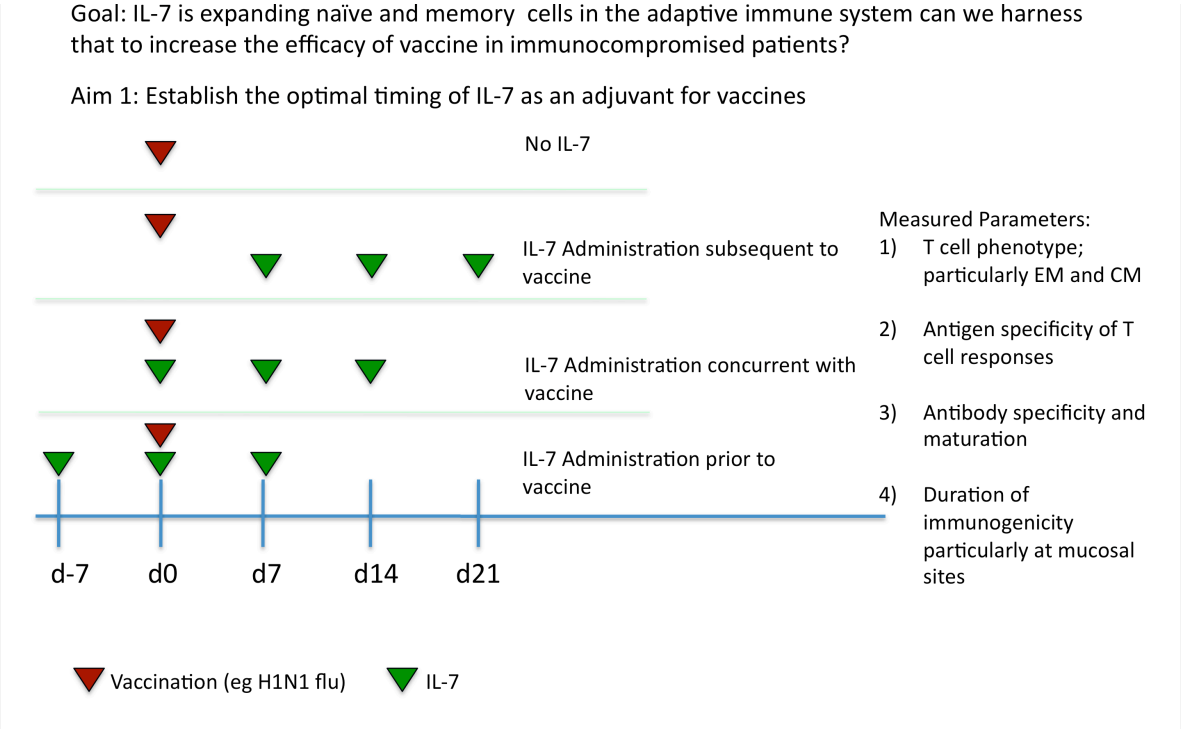


Figure 6-2: A working hypothesis demonstrating why effective ART treatment is crucial for rsIL-7gly efficacy in the macaque model. Undividing resting T cells are shown in blue, Ki67+ cells are shown in green and SIV+ cells are shown in red.

Figure 6-3: Assessing IL-7 as a vaccine adjuvant



BIBLIOGRAPHY

1. **Abbate, I., F. Dianzani, and M. R. Capobianchi.** 2000. Activation of signal transduction and apoptosis in healthy lymphomonocytes exposed to bystander HIV-1-infected cells. Clin Exp Immunol **122:**374-80.

2. **Abrams, D., Y. Levy, M. H. Losso, A. Babiker, G. Collins, D. A. Cooper, J. Darbyshire, S. Emery, L. Fox, F. Gordin, H. C. Lane, J. D. Lundgren, R. Mitsuyasu, J. D. Neaton, A. Phillips, J. P. Routy, G. Tambussi, and D. Wentworth.** 2009. Interleukin-2 therapy in patients with HIV infection. N Engl J Med **361:**1548-59.

3. **Ahmed, R. K., G. Biberfeld, and R. Thorstensson.** 2005. Innate immunity in experimental SIV infection and vaccination. Mol Immunol **42:**251-8.

4. **Aiuti, F., and I. Mezzaroma.** 2006. Failure to reconstitute CD4+ T-cells despite suppression of HIV replication under HAART. AIDS Rev **8:**88-97.

5. **Albuquerque, A., R. Foxall, C. Cortesão, R. Soares, M. Doroana, A. Ribeiro, M. Lucas, F. Antunes, R. Victorino, and A. Sousa.** 2007. Low CD4 T-cell counts despite low levels of circulating HIV: insights from the comparison of HIV-1 infected patients with a discordant response to antiretroviral therapy to patients with untreated advanced HIV-2 disease. Clin Immunol **125:**67-75.

6. **Allman, D., and J. P. Miller.** 2003. Common lymphoid progenitors, early B-lineage precursors, and IL-7: characterizing the trophic and instructive signals underlying early B cell development. Immunol Res **27:**131-40.

7. **Alpdogan, O., and M. R. van den Brink.** 2005. IL-7 and IL-15: therapeutic cytokines for immunodeficiency. Trends Immunol **26:**56-64.

8. **Appay, V., and D. Sauce.** 2008. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol **214:**231-41.

9. **Appleman, L. J., A. A. van Puijenbroek, K. M. Shu, L. M. Nadler, and V. A. Boussiotis.** 2002. CD28 costimulation mediates down-regulation of p27kip1 and cell cycle progression by activation of the PI3K/PKB signaling pathway in primary human T cells. J Immunol **168:**2729-36.

10. **Bachmann, M. F., P. Wolint, K. Schwarz, P. Jager, and A. Oxenius.** 2005. Functional properties and lineage relationship of CD8+ T cell subsets identified by expression of IL-7 receptor alpha and CD62L. J Immunol **175:**4686-96.

11. **Barata, J. T., A. A. Cardoso, and V. A. Boussiotis.** 2005. Interleukin-7 in T-cell acute lymphoblastic leukemia: an extrinsic factor supporting leukemogenesis? Leuk Lymphoma **46:**483-95.

12. **Barata, J. T., A. A. Cardoso, L. M. Nadler, and V. A. Boussiotis.** 2001. Interleukin-7 promotes survival and cell cycle progression of T-cell acute lymphoblastic leukemia cells by down-regulating the cyclin-dependent kinase inhibitor p27(kip1). Blood **98:**1524-31.

13. **Barata, J. T., A. Silva, J. G. Brandao, L. M. Nadler, A. A. Cardoso, and V. A. Boussiotis.** 2004. Activation of PI3K is indispensable for interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. J Exp Med **200:**659-69.

14. **Barouch, D.** 2008. Challenges in the development of an HIV-1 vaccine. Nature **455:**613-9.

15. **Barouch, D., T. Fu, D. Montefiori, M. Lewis, J. Shiver, and N. Letvin.** 2001. Vaccine-elicited immune responses prevent clinical AIDS in SHIV(89.6P)-infected rhesus monkeys. Immunol Lett **79:**57-61.

16. **Barouch, D., S. Santra, J. Schmitz, M. Kuroda, T. Fu, W. Wagner, M. Bilska, A. Craiu, X. Zheng, G. Krivulka, K. Beaudry, M. Lifton, C. Nickerson, W. Trigona, K. Punt, D. Freed, L. Guan, S. Dubey, D. Casimiro, A. Simon, M. Davies, M. Chastain, T. Strom, R. Gelman, D. Montefiori, M. Lewis, E. Emini, J. Shiver, and N. Letvin.** 2000. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science **290:**486-92.

17. **Beaumier, C. M., L. D. Harris, S. Goldstein, N. R. Klatt, S. Whitted, J. McGinty, C. Apetrei, I. Pandrea, V. M. Hirsch, and J. M. Brenchley.** 2009. CD4 downregulation by memory CD4+ T cells in vivo renders African green monkeys resistant to progressive SIVagm infection. Nat Med **15:**879-85.

18. **Becker, T., E. Wherry, D. Boone, K. Murali-Krishna, R. Antia, A. Ma, and R. Ahmed.** 2002. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. J Exp Med **195:**1541-8.

19. **Becknell, B., and M. Caligiuri.** 2005. Interleukin-2, interleukin-15, and their roles in human natural killer cells. Adv Immunol **86:**209-39.

20. **Benczik, M., and S. Gaffen.** 2004. The interleukin (IL)-2 family cytokines: survival and proliferation signaling pathways in T lymphocytes. Immunol Invest **33:**109-42.

21. **Benicchi, T., C. Ghidini, A. Re, C. Cattaneo, S. Casari, L. Caimi, G. Rossi, and L. Imberti.** 2005. T-cell immune reconstitution after hematopoietic stem cell transplantation for HIV-associated lymphoma. Transplantation **80:**673-82.

22. **Benito, J. M., M. Lopez, S. Lozano, J. Gonzalez-Lahoz, and V. Soriano.** 2008. Down-regulation of interleukin-7 receptor (CD127) in HIV infection is associated with T cell activation and is a main factor influencing restoration of CD4(+) cells after antiretroviral therapy. J Infect Dis **198:**1466-73.

23. **Beq, S., F. Bugault, J. H. Colle, O. Lambotte, J. F. Delfraissy, and J. Theze.** 2005. Anti-retroviral therapy in HIV-infected patients: in vitro effects of AZT and saquinavir on the response of CD4 and CD8 lymphocytes to interleukin-7. Eur Cytokine Netw **16:**293-9.

24. **Beq, S., M. T. Nugeyre, R. Ho Tsong Fang, D. Gautier, R. Legrand, N. Schmitt, J. Estaquier, F. Barre-Sinoussi, B. Hurtrel, R. Cheynier, and N. Israel.** 2006. IL-7 induces immunological improvement in SIV-infected rhesus macaques under antiviral therapy. J Immunol **176:**914-22.

25. **Beq, S., S. Rozlan, D. Gautier, R. Parker, V. Mersseman, C. Schilte, B. Assouline, I. Rance, P. Lavedan, M. Morre, and R. Cheynier.** 2009. Injection of glycosylated recombinant simian IL-7 provokes rapid and massive T-cell homing in rhesus macaques. Blood.

26. **Bhattacharyya, A., S. Pathak, S. Datta, S. Chattopadhyay, J. Basu, and M. Kundu.** 2002. Mitogen-activated protein kinases and nuclear factor-kappaB regulate Helicobacter pylori-mediated interleukin-8 release from macrophages. Biochem J **368:**121-9.

27. **Bostik, P., G. L. Dodd, F. Villinger, A. E. Mayne, and A. A. Ansari.** 2004. Dysregulation of the polo-like kinase pathway in CD4+ T cells is characteristic of pathogenic simian immunodeficiency virus infection. J Virol **78:**1464-72.

28. **Bostik, P., P. Wu, G. L. Dodd, F. Villinger, A. E. Mayne, V. Bostik, B. D. Grimm, D. Robinson, H. J. Kung, and A. A. Ansari.** 2001. Identification of protein kinases dysregulated in CD4(+) T cells in pathogenic versus apathogenic simian immunodeficiency virus infection. J Virol **75:**11298-306.

29. **Bovolenta, C., L. Camorali, A. L. Lorini, S. Ghezzi, E. Vicenzi, A. Lazzarin, and G. Poli.** 1999. Constitutive activation of STATs upon in vivo human immunodeficiency virus infection. Blood **94:**4202-9.

30. **Bovolenta, C., L. Camorali, M. Mauri, S. Ghezzi, S. Nozza, G. Tambussi, A. Lazzarin, and G. Poli.** 2001. Expression and activation of a C-terminal truncated isoform of STAT5 (STAT5 Delta) following interleukin 2 administration or AZT monotherapy in HIV-infected individuals. Clin Immunol **99:**75-81.

31. **Bovolenta, C., E. Pilotti, M. Mauri, B. Panzeri, M. Sassi, P. Dall'Aglio, U. Bertazzoni, G. Poli, and C. Casoli.** 2002. Retroviral interference on STAT activation in individuals coinfected with human T cell leukemia virus type 2 and HIV-1. J Immunol **169:**4443-9.

32. **Boyer, J., T. Robinson, M. Kutzler, G. Vansant, D. Hokey, S. Kumar, R. Parkinson, L. Wu, M. Sidhu, G. Pavlakis, B. Felber, C. Brown, P. Silvera, M. Lewis, J. Monforte, T. Waldmann, J. Eldridge, and D.**

Weiner. 2007. Protection against simian/human immunodeficiency virus (SHIV) 89.6P in macaques after coimmunization with SHIV antigen and IL-15 plasmid. Proc Natl Acad Sci U S A **104:**18648-53.

33. **Boyman, O., J. Purton, C. Surh, and J. Sprent.** 2007. Cytokines and T-cell homeostasis. Curr Opin Immunol **19:**320-6.

34. **Bradley, L. M., L. Haynes, and S. L. Swain.** 2005. IL-7: maintaining T-cell memory and achieving homeostasis. Trends Immunol **26:**172-6.

35. **Brenchley, J., D. Price, T. Schacker, T. Asher, G. Silvestri, S. Rao, Z. Kazzaz, E. Bornstein, O. Lambotte, D. Altmann, B. Blazar, B. Rodriguez, L. Teixeira-Johnson, A. Landay, J. Martin, F. Hecht, L. Picker, M. Lederman, S. Deeks, and D. Douek.** 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **12:**1365-71.

36. **Brenchley, J. M., T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek.** 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med **200:**749-59.

37. **Budagian, V., E. Bulanova, Z. Orinska, T. Pohl, E. Borden, R. Silverman, and S. Bulfone-Paus.** 2004. Reverse signaling through membrane-bound interleukin-15. J Biol Chem **279:**42192-201.

38. **Budagian, V., E. Bulanova, R. Paus, and S. Bulfone-Paus.** 2006. IL-15/IL-15 receptor biology: a guided tour through an expanding universe. Cytokine Growth Factor Rev **17:**259-80.

39. **Bulanova, E., V. Budagian, E. Duitman, Z. Orinska, H. Krause, R. Rückert, N. Reiling, and S. Bulfone-Paus.** 2007. Soluble Interleukin IL-15Ralpha is generated by alternative splicing or proteolytic cleavage and forms functional complexes with IL-15. J Biol Chem **282:**13167-79.

40. **Bulfone-PauS, S., E. Bulanova, T. Pohl, V. Budagian, H. Durkop, R. Ruckert, U. Kunzendorf, R. Paus, and H. Krause.** 1999. Death

deflected: IL-15 inhibits TNF-alpha-mediated apoptosis in fibroblasts by TRAF2 recruitment to the IL-15Ralpha chain. FASEB J **13:**1575-85.

41. **Burkett, P., R. Koka, M. Chien, S. Chai, F. Chan, A. Ma, and D. Boone.** 2003. IL-15R alpha expression on CD8+ T cells is dispensable for T cell memory. Proc Natl Acad Sci U S A **100:**4724-9.

42. **Cagigi, A., L. Du, L. V. Dang, S. Grutzmeier, A. Atlas, F. Chiodi, Q. Pan-Hammarstrom, and A. Nilsson.** 2009. CD27(-) B-cells produce class switched and somatically hyper-mutated antibodies during chronic HIV-1 infection. PLoS One **4:**e5427.

43. **Casimiro, D. R., F. Wang, W. A. Schleif, X. Liang, Z. Q. Zhang, T. W. Tobery, M. E. Davies, A. B. McDermott, D. H. O'Connor, A. Fridman, A. Bagchi, L. G. Tussey, A. J. Bett, A. C. Finnefrock, T. M. Fu, A. Tang, K. A. Wilson, M. Chen, H. C. Perry, G. J. Heidecker, D. C. Freed, A. Carella, K. S. Punt, K. J. Sykes, L. Huang, V. I. Ausensi, M. Bachinsky, U. Sadasivan-Nair, D. I. Watkins, E. A. Emini, and J. W. Shiver.** 2005. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. J Virol **79:**15547-55.

44. **Catalfamo, M., M. Di Mascio, Z. Hu, S. Srinivasula, V. Thaker, J. Adelsberger, A. Rupert, M. Baseler, Y. Tagaya, G. Roby, C. Rehm, D. Follmann, and H. C. Lane.** 2008. HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T cells. Proc Natl Acad Sci U S A **105:**19851-6.

45. **Chang, T. L., A. Mosoian, R. Pine, M. E. Klotman, and J. P. Moore.** 2002. A soluble factor(s) secreted from CD8(+) T lymphocytes inhibits human immunodeficiency virus type 1 replication through STAT1 activation. J Virol **76:**569-81.

46. **Chen, C. Y., D. Huang, R. C. Wang, L. Shen, G. Zeng, S. Yao, Y. Shen, L. Halliday, J. Fortman, M. McAllister, J. Estep, R. Hunt, D. Vasconcelos, G. Du, S. A. Porcelli, M. H. Larsen, W. R. Jacobs, Jr., B. F. Haynes, N. L. Letvin, and Z. W. Chen.** 2009. A critical role for CD8

T cells in a nonhuman primate model of tuberculosis. PLoS Pathog **5:**e1000392.

47. **Chen, D., V. Heath, A. O'Garra, J. Johnston, and M. McMahon.** 1999. Sustained activation of the raf-MEK-ERK pathway elicits cytokine unresponsiveness in T cells. J Immunol **163:**5796-805.

48. **Chen, W., M. S. Ford, K. J. Young, and L. Zhang.** 2004. The role and mechanisms of double negative regulatory T cells in the suppression of immune responses. Cell Mol Immunol **1:**328-35.

49. **Chong, S. Y., M. A. Egan, M. A. Kutzler, S. Megati, A. Masood, V. Roopchard, D. Garcia-Hand, D. C. Montefiori, J. Quiroz, M. Rosati, E. B. Schadeck, J. D. Boyer, G. N. Pavlakis, D. B. Weiner, M. Sidhu, J. H. Eldridge, and Z. R. Israel.** 2007. Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. Vaccine **25:**4967-82.

50. **Chung, T. W., Y. C. Lee, and C. H. Kim.** 2004. Hepatitis B viral HBx induces matrix metalloproteinase-9 gene expression through activation of ERK and PI-3K/AKT pathways: involvement of invasive potential. FASEB J **18:**1123-5.

51. **Cline, A. N., J. W. Bess, M. Piatak, Jr., and J. D. Lifson.** 2005. Highly sensitive SIV plasma viral load assay: practical considerations, realistic performance expectations, and application to reverse engineering of vaccines for AIDS. J Med Primatol **34:**303-12.

52. **Cole, K. S., J. L. Rowles, B. A. Jagerski, M. Murphey-Corb, T. Unangst, J. E. Clements, J. Robinson, M. S. Wyand, R. C. Desrosiers, and R. C. Montelaro.** 1997. Evolution of envelope-specific antibody responses in monkeys experimentally infected or immunized with simian immunodeficiency virus and its association with the development of protective immunity. J Virol **71:**5069-79.

53. **Colle, J. H., J. L. Moreau, A. Fontanet, O. Lambotte, M. Joussemet, J. F. Delfraissy, and J. Theze.** 2006. CD127 expression and regulation are

altered in the memory CD8 T cells of HIV-infected patients--reversal by highly active anti-retroviral therapy (HAART). Clin Exp Immunol **143:**398-403.

54. **Colle, J. H., J. L. Moreau, A. Fontanet, O. Lambotte, M. Joussemet, S. Jacod, J. F. Delfraissy, and J. Theze.** 2006. Regulatory dysfunction of the interleukin-7 receptor in CD4 and CD8 lymphocytes from HIV-infected patients--effects of antiretroviral therapy. J Acquir Immune Defic Syndr **42:**277-85.

55. **Colombetti, S., F. Levy, and L. Chapatte.** 2009. IL-7 adjuvant treatment enhances long-term tumor-antigen-specific CD8+ T-cell responses after immunization with recombinant lentivector. Blood **113:**6629-37.

56. **Crawley, J. B., J. Willcocks, and B. M. Foxwell.** 1996. Interleukin-7 induces T cell proliferation in the absence of Erk/MAP kinase activity. Eur J Immunol **26:**2717-23.

57. **Crotti, A., M. Lusic, R. Lupo, P. M. Lievens, E. Liboi, G. Della Chiara, M. Tinelli, A. Lazzarin, B. K. Patterson, M. Giacca, C. Bovolenta, and G. Poli.** 2007. Naturally occurring C-terminally truncated STAT5 is a negative regulator of HIV-1 expression. Blood **109:**5380-9.

58. **d'Ettorre, G., G. Forcina, M. Andreotti, L. Sarmati, L. Palmisano, M. Andreoni, S. Vella, C. M. Mastroianni, and V. Vullo.** 2004. Interleukin-15 production by monocyte-derived dendritic cells and T cell proliferation in HIV-infected patients with discordant response to highly active antiretroviral therapy. Clin Exp Immunol **135:**280-5.

59. **D'Orsogna, L. J., R. G. Krueger, E. J. McKinnon, and M. A. French.** 2007. Circulating memory B-cell subpopulations are affected differently by HIV infection and antiretroviral therapy. AIDS **21:**1747-52.

60. **Davey, R. J., R. Murphy, F. Graziano, S. Boswell, A. Pavia, M. Cancio, J. Nadler, D. Chaitt, R. Dewar, D. Sahner, A. Duliege, W. Capra, W. Leong, M. Giedlin, H. Lane, and J. Kahn.** 2000. Immunologic and virologic effects of subcutaneous interleukin 2 in combination with antiretroviral therapy: A randomized controlled trial. JAMA **284:**183-9.

61. **Davey, R. T., Jr., D. G. Chaitt, J. M. Albert, S. C. Piscitelli, J. A. Kovacs, R. E. Walker, J. Falloon, M. A. Polis, J. A. Metcalf, H. Masur, R. Dewar, M. Baseler, G. Fyfe, M. A. Giedlin, and H. C. Lane.** 1999. A randomized trial of high- versus low-dose subcutaneous interleukin-2 outpatient therapy for early human immunodeficiency virus type 1 infection. J Infect Dis **179:**849-58.

62. **De Boer, R. J., H. Mohri, D. D. Ho, and A. S. Perelson.** 2003. Turnover rates of B cells, T cells, and NK cells in simian immunodeficiency virus-infected and uninfected rhesus macaques. J Immunol **170:**2479-87.

63. **Delobel, P., M. T. Nugeyre, M. Cazabat, K. Sandres-Saune, C. Pasquier, L. Cuzin, B. Marchou, P. Massip, R. Cheynier, F. Barre-Sinoussi, J. Izopet, and N. Israel.** 2006. Naive T-cell depletion related to infection by X4 human immunodeficiency virus type 1 in poor immunological responders to highly active antiretroviral therapy. J Virol **80:**10229-36.

64. **Delobel, P., K. Sandres-Saune, M. Cazabat, C. Pasquier, B. Marchou, P. Massip, and J. Izopet.** 2005. R5 to X4 switch of the predominant HIV-1 population in cellular reservoirs during effective highly active antiretroviral therapy. J Acquir Immune Defic Syndr **38:**382-92.

65. **Dereuddre-Bosquet, N., B. Vaslin, B. Delache, P. Brochard, P. Clayette, C. Aubenque, M. Morre, B. Assouline, and R. Le Grand.** 2007. Rapid modifications of peripheral T-cell subsets that express CD127 in macaques treated with recombinant IL-7. J Med Primatol **36:**228-37.

66. **Dion, M., R. Bordi, J. Zeidan, R. Asaad, M. Boulassel, J. Routy, M. Lederman, R. Sekaly, and R. Cheynier.** 2007. Slow disease progression and robust therapy-mediated CD4+ T-cell recovery are associated with efficient thymopoiesis during HIV-1 infection. Blood **109:**2912-20.

67. **Dion, M., J. Poulin, R. Bordi, M. Sylvestre, R. Corsini, N. Kettaf, A. Dalloul, M. Boulassel, P. Debré, J. Routy, Z. Grossman, R. Sékaly, and R. Cheynier.** 2004. HIV infection rapidly induces and maintains a substantial suppression of thymocyte proliferation. Immunity **21:**757-68.

68. **Douek, D., R. McFarland, P. Keiser, E. Gage, J. Massey, B. Haynes, M. Polis, A. Haase, M. Feinberg, J. Sullivan, B. Jamieson, J. Zack, L. Picker, and R. Koup.** 1998. Changes in thymic function with age and during the treatment of HIV infection. Nature **396:**690-5.

69. **Douek, D., L. Picker, and R. Koup.** 2003. T cell dynamics in HIV-1 infection. Annu Rev Immunol **21:**265-304.

70. **Dubois, S., J. Mariner, T. Waldmann, and Y. Tagaya.** 2002. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. Immunity **17:**537-47.

71. **Ducrey-Rundquist, O., M. Guyader, and D. Trono.** 2002. Modalities of interleukin-7-induced human immunodeficiency virus permissiveness in quiescent T lymphocytes. J Virol **76:**9103-11.

72. **Dumont-Girard, F., E. Roux, R. van Lier, G. Hale, C. Helg, B. Chapuis, M. Starobinski, and E. Roosnek.** 1998. Reconstitution of the T-cell compartment after bone marrow transplantation: restoration of the repertoire by thymic emigrants. Blood **92:**4464-71.

73. **El-Sadr, W. M., J. D. Lundgren, J. D. Neaton, F. Gordin, D. Abrams, R. C. Arduino, A. Babiker, W. Burman, N. Clumeck, C. J. Cohen, D. Cohn, D. Cooper, J. Darbyshire, S. Emery, G. Fatkenheuer, B. Gazzard, B. Grund, J. Hoy, K. Klingman, M. Losso, N. Markowitz, J. Neuhaus, A. Phillips, and C. Rappoport.** 2006. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med **355:**2283-96.

74. **Erickson, S., O. Sangfelt, J. Castro, M. Heyman, S. Einhorn, and D. Grander.** 1999. Interferon-alpha inhibits proliferation in human T lymphocytes by abrogation of interleukin 2-induced changes in cell cycle-regulatory proteins. Cell Growth Differ **10:**575-82.

75. **Estes, J. D., S. N. Gordon, M. Zeng, A. M. Chahroudi, R. M. Dunham, S. I. Staprans, C. S. Reilly, G. Silvestri, and A. T. Haase.** 2008. Early resolution of acute immune activation and induction of PD-1 in SIV-infected sooty mangabeys distinguishes nonpathogenic from pathogenic infection in rhesus macaques. J Immunol **180:**6798-807.

76. **Eyrich, M., T. Croner, C. Leiler, P. Lang, P. Bader, T. Klingebiel, D. Niethammer, and P. Schlegel.** 2002. Distinct contributions of CD4(+) and CD8(+) naive and memory T-cell subsets to overall T-cell-receptor repertoire complexity following transplantation of T-cell-depleted CD34-selected hematopoietic progenitor cells from unrelated donors. Blood **100:**1915-8.

77. **Eyrich, M., G. Wollny, N. Tzaribaschev, K. Dietz, D. Brügger, P. Bader, P. Lang, K. Schilbach, B. Winkler, D. Niethammer, and P. Schlegel.** 2005. Onset of thymic recovery and plateau of thymic output are differentially regulated after stem cell transplantation in children. Biol Blood Marrow Transplant **11:**194-205.

78. **Fahey, J., J. Taylor, B. Manna, P. Nishanian, N. Aziz, J. Giorgi, and R. Detels.** 1998. Prognostic significance of plasma markers of immune activation, HIV viral load and CD4 T-cell measurements. AIDS **12:**1581-90.

79. **Falco, V., Q. Jordano, M. J. Cruz, O. Len, E. Ribera, M. Campins, M. Crespo, I. Ocana, M. J. Rodrigo, and A. Pahissa.** 2006. Serological response to pneumococcal vaccination in HAART-treated HIV-infected patients: one year follow-up study. Vaccine **24:**2567-74.

80. **Farel, C., D. Chaitt, B. Hahn, J. Tavel, J. Kovacs, M. Polis, H. Masur, D. Follmann, H. Lane, and R. J. Davey.** 2004. Induction and maintenance therapy with intermittent interleukin-2 in HIV-1 infection. Blood **103:**3282-6.

81. **Feldman, R. L., and K. Nickell.** 2007. Avian influenza: potential impact on sub-Saharan military populations with high rates of human immunodeficiency virus/acquired immunodeficiency syndrome. Mil Med **172:**753-8.

82. **Finzi, D., S. Plaeger, and C. Dieffenbach.** 2006. Defective virus drives human immunodeficiency virus infection, persistence, and pathogenesis. Clin Vaccine Immunol **13:**715-21.

83. **Fleming, H. E., and C. J. Paige.** 2002. Cooperation between IL-7 and the pre-B cell receptor: a key to B cell selection. Semin Immunol **14:**423-30.

84. **Fluur, C., A. De Milito, T. Fry, N. Vivar, L. Eidsmo, A. Atlas, C. Federici, P. Matarrese, M. Logozzi, E. Rajnavölgyi, C. Mackall, S. Fais, F. Chiodi, and B. Rethi.** 2007. Potential role for IL-7 in Fas-mediated T cell apoptosis during HIV infection. J Immunol **178:**5340-50.

85. **Ford, M. S., K. J. Young, Z. Zhang, P. S. Ohashi, and L. Zhang.** 2002. The immune regulatory function of lymphoproliferative double negative T cells in vitro and in vivo. J Exp Med **196:**261-7.

86. **Ford, M. S., Z. X. Zhang, W. Chen, and L. Zhang.** 2006. Double-negative T regulatory cells can develop outside the thymus and do not mature from CD8+ T cell precursors. J Immunol **177:**2803-9.

87. **Francois, F., and M. E. Klotman.** 2003. Phosphatidylinositol 3-kinase regulates human immunodeficiency virus type 1 replication following viral entry in primary CD4+ T lymphocytes and macrophages. J Virol **77:**2539-49.

88. **Fruman, D. A.** 2004. Phosphoinositide 3-kinase and its targets in B-cell and T-cell signaling. Curr Opin Immunol **16:**314-20.

89. **Fry, T., and C. Mackall.** 2002. Interleukin-7 and immunorestoration in HIV: beyond the thymus. J Hematother Stem Cell Res **11:**803-7.

90. **Fry, T., and C. Mackall.** 2001. What limits immune reconstitution in HIV infection? Divergent tools converge on thymic function. AIDS **15:**1881-2.

91. **Fry, T. J., and C. L. Mackall.** 2002. Interleukin-7: from bench to clinic. Blood **99:**3892-904.

92. **Fry, T. J., M. Moniuszko, S. Creekmore, S. J. Donohue, D. C. Douek, S. Giardina, T. T. Hecht, B. J. Hill, K. Komschlies, J. Tomaszewski, G. Franchini, and C. L. Mackall.** 2003. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. Blood **101:**2294-9.

93. **Gattinoni, L., S. E. Finkelstein, C. A. Klebanoff, P. A. Antony, D. C. Palmer, P. J. Spiess, L. N. Hwang, Z. Yu, C. Wrzesinski, D. M.**

Heimann, C. D. Surh, S. A. Rosenberg, and N. P. Restifo. 2005. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med **202:**907-12.

94. **Geiselhart, L. A., C. A. Humphries, T. A. Gregorio, S. Mou, J. Subleski, and K. L. Komschlies.** 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:**3019-27.

95. **Giliani, S., L. Mori, G. de Saint Basile, F. Le Deist, C. Rodriguez-Perez, C. Forino, E. Mazzolari, S. Dupuis, R. Elhasid, A. Kessel, C. Galambrun, J. Gil, A. Fischer, A. Etzioni, and L. D. Notarangelo.** 2005. Interleukin-7 receptor alpha (IL-7Ralpha) deficiency: cellular and molecular bases. Analysis of clinical, immunological, and molecular features in 16 novel patients. Immunol Rev **203:**110-26.

96. **Goldrath, A., and M. Bevan.** 1999. Selecting and maintaining a diverse T-cell repertoire. Nature **402:**255-62.

97. **Goldstein, S., C. Brown, H. Dehghani, J. Lifson, and V. Hirsch.** 2000. Intrinsic susceptibility of rhesus macaque peripheral CD4(+) T cells to simian immunodeficiency virus in vitro is predictive of in vivo viral replication. J Virol **74:**9388-95.

98. **Gomez, C., and T. Hope.** 2005. The ins and outs of HIV replication. Cell Microbiol **7:**621-6.

99. **Greene, W., and B. Peterlin.** 2002. Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. Nat Med **8:**673-80.

100. **Grossman, Z., M. Meier-Schellersheim, W. E. Paul, and L. J. Picker.** 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med **12:**289-95.

101. **Hart, M., A. Steel, S. A. Clark, G. Moyle, M. Nelson, D. C. Henderson, R. Wilson, F. Gotch, B. Gazzard, and P. Kelleher.** 2007. Loss of discrete memory B cell subsets is associated with impaired immunization

responses in HIV-1 infection and may be a risk factor for invasive pneumococcal disease. J Immunol **178:**8212-20.

102. **Haynes, B., and R. Shattock.** 2008. Critical issues in mucosal immunity for HIV-1 vaccine development. J Allergy Clin Immunol **122:**3-9; quiz 10-1.

103. **Hazenberg, M., J. Stuart, S. Otto, J. Borleffs, C. Boucher, R. de Boer, F. Miedema, and D. Hamann.** 2000. T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). Blood **95:**249-55.

104. **Hirsch, V., D. Adger-Johnson, B. Campbell, S. Goldstein, C. Brown, W. R. Elkins, and D. C. Montefiori.** 1997. A molecularly cloned, pathogenic, neutralization-resistant simian immunodeficiency virus, SIVsmE543-3. J Virol **71:**1608-20.

105. **Hirsch, V., S. Santra, S. Goldstein, R. Plishka, A. Buckler-White, A. Seth, I. Ourmanov, C. Brown, R. Engle, D. Montefiori, J. Glowczwskie, K. Kunstman, S. Wolinsky, and N. Letvin.** 2004. Immune failure in the absence of profound CD4+ T-lymphocyte depletion in simian immunodeficiency virus-infected rapid progressor macaques. J Virol **78:**275-84.

106. **Hirsch, V. M., P. M. Zack, A. P. Vogel, and P. R. Johnson.** 1991. Simian immunodeficiency virus infection of macaques: end-stage disease is characterized by widespread distribution of proviral DNA in tissues. J Infect Dis **163:**976-88.

107. **Hofmeister, R., A. R. Khaled, N. Benbernou, E. Rajnavolgyi, K. Muegge, and S. K. Durum.** 1999. Interleukin-7: physiological roles and mechanisms of action. Cytokine Growth Factor Rev **10:**41-60.

108. **Holterman, L., H. Niphuis, P. J. ten Haaft, J. Goudsmit, G. Baskin, and J. L. Heeney.** 1999. Specific passage of simian immunodeficiency virus from end-stage disease results in accelerated progression to AIDS in rhesus macaques. J Gen Virol **80 (Pt 12):**3089-97.

109. **Horton, H., T. U. Vogel, D. K. Carter, K. Vielhuber, D. H. Fuller, T. Shipley, J. T. Fuller, K. J. Kunstman, G. Sutter, D. C. Montefiori, V. Erfle, R. C. Desrosiers, N. Wilson, L. J. Picker, S. M. Wolinsky, C. Wang, D. B. Allison, and D. I. Watkins.** 2002. Immunization of rhesus macaques with a DNA prime/modified vaccinia virus Ankara boost regimen induces broad simian immunodeficiency virus (SIV)-specific T-cell responses and reduces initial viral replication but does not prevent disease progression following challenge with pathogenic SIVmac239. J Virol **76:**7187-202.

110. **Hossain, M. S., H. Takimoto, T. Ninomiya, H. Yoshida, K. Kishihara, G. Matsuzaki, G. Kimura, and K. Nomoto.** 2000. Characterization of CD4- CD8- CD3+ T-cell receptor-alphabeta+ T cells in murine cytomegalovirus infection. Immunology **101:**19-29.

111. **Hryniewicz, A., D. A. Price, M. Moniuszko, A. Boasso, Y. Edghill-Spano, S. M. West, D. Venzon, M. Vaccari, W. P. Tsai, E. Tryniszewska, J. Nacsa, F. Villinger, A. A. Ansari, C. J. Trindade, M. Morre, D. Brooks, P. Arlen, H. J. Brown, C. M. Kitchen, J. A. Zack, D. C. Douek, G. M. Shearer, M. G. Lewis, R. A. Koup, and G. Franchini.** 2007. Interleukin-15 but not interleukin-7 abrogates vaccine-induced decrease in virus level in simian immunodeficiency virus mac251-infected macaques. J Immunol **178:**3492-504.

112. **Imamichi, H., I. Sereti, and H. Lane.** 2008. IL-15 acts as a potent inducer of CD4(+)CD25(hi) cells expressing FOXP3. Eur J Immunol **38:**1621-30.

113. **Isgro, A., W. Leti, W. De Santis, M. Marziali, A. Esposito, C. Fimiani, G. Luzi, M. Pinti, A. Cossarizza, F. Aiuti, and I. Mezzaroma.** 2008. Altered clonogenic capability and stromal cell function characterize bone marrow of HIV-infected subjects with low CD4+ T cell counts despite viral suppression during HAART. Clin Infect Dis **46:**1902-10.

114. **Jacobson, E. L., F. Pilaro, and K. A. Smith.** 1996. Rational interleukin 2 therapy for HIV positive individuals: daily low doses enhance immune function without toxicity. Proc Natl Acad Sci U S A **93:**10405-10.

115. **Jaleco, S., L. Swainson, V. Dardalhon, M. Burjanadze, S. Kinet, and N. Taylor.** 2003. Homeostasis of naive and memory CD4+ T cells: IL-2 and IL-7 differentially regulate the balance between proliferation and Fas-mediated apoptosis. J Immunol **171:**61-8.

116. **Jiang, Q., W. Li, F. Aiello, R. Mazzucchelli, B. Asefa, A. Khaled, and S. Durum.** 2005. Cell biology of IL-7, a key lymphotrophin. Cytokine Growth Factor Rev **16:**513-33.

117. **Jiang, Q., W. Q. Li, R. R. Hofmeister, H. A. Young, D. R. Hodge, J. R. Keller, A. R. Khaled, and S. K. Durum.** 2004. Distinct regions of the interleukin-7 receptor regulate different Bcl2 family members. Mol Cell Biol **24:**6501-13.

118. **Jiang, W., M. M. Lederman, R. J. Mohner, B. Rodriguez, T. M. Nedrich, C. V. Harding, and S. F. Sieg.** 2008. Impaired naive and memory B-cell responsiveness to TLR9 stimulation in human immunodeficiency virus infection. J Virol **82:**7837-45.

119. **Johnson, P. R., B. C. Schnepp, M. J. Connell, D. Rohne, S. Robinson, G. R. Krivulka, C. I. Lord, R. Zinn, D. C. Montefiori, N. L. Letvin, and K. R. Clark.** 2005. Novel adeno-associated virus vector vaccine restricts replication of simian immunodeficiency virus in macaques. J Virol **79:**955-65.

120. **Johnson, R. A., X. L. Ma, A. D. Yurochko, and E. S. Huang.** 2001. The role of MKK1/2 kinase activity in human cytomegalovirus infection. J Gen Virol **82:**493-7.

121. **Kaiser, P., B. Joos, B. Niederost, R. Weber, H. F. Gunthard, and M. Fischer.** 2007. Productive human immunodeficiency virus type 1 infection in peripheral blood predominantly takes place in CD4/CD8 double-negative T lymphocytes. J Virol **81:**9693-706.

122. **Kang, J., and S. D. Der.** 2004. Cytokine functions in the formative stages of a lymphocyte's life. Curr Opin Immunol **16:**180-90.

123. **Kaur, A., R. M. Grant, R. E. Means, H. McClure, M. Feinberg, and R. P. Johnson.** 1998. Diverse host responses and outcomes following simian

immunodeficiency virus SIVmac239 infection in sooty mangabeys and rhesus macaques. J Virol **72:**9597-611.

124. **Kawada, M., T. Tsukamoto, H. Yamamoto, N. Iwamoto, K. Kurihara, A. Takeda, C. Moriya, H. Takeuchi, H. Akari, and T. Matano.** 2008. Gag-specific cytotoxic T-lymphocyte-based control of primary simian immunodeficiency virus replication in a vaccine trial. J Virol **82:**10199-206.

125. **Khaled, A., W. Li, J. Huang, T. Fry, A. Khaled, C. Mackall, K. Muegge, H. Young, and S. Durum.** 2002. Bax deficiency partially corrects interleukin-7 receptor alpha deficiency. Immunity **17:**561-73.

126. **Khaled, A. R., D. V. Bulavin, C. Kittipatarin, W. Q. Li, M. Alvarez, K. Kim, H. A. Young, A. J. Fornace, and S. K. Durum.** 2005. Cytokine-driven cell cycling is mediated through Cdc25A. J Cell Biol **169:**755-63.

127. **Kim, T. S., S. W. Chung, and S. Y. Hwang.** 2000. Augmentation of antitumor immunity by genetically engineered fibroblast cells to express both B7.1 and interleukin-7. Vaccine **18:**2886-94.

128. **Kittipatarin, C., W. Li, D. Bulavin, S. Durum, and A. Khaled.** 2006. Cell cycling through Cdc25A: transducer of cytokine proliferative signals. Cell Cycle **5:**907-12.

129. **Klimas, N., A. Koneru, and M. Fletcher.** 2008. Overview of HIV. Psychosom Med **70:**523-30.

130. **Klonowski, K. D., K. J. Williams, A. L. Marzo, and L. Lefrancois.** 2006. Cutting edge: IL-7-independent regulation of IL-7 receptor alpha expression and memory CD8 T cell development. J Immunol **177:**4247-51.

131. **Koesters, S. A., J. B. Alimonti, C. Wachihi, L. Matu, O. Anzala, J. Kimani, J. E. Embree, F. A. Plummer, and K. R. Fowke.** 2006. IL-7Ralpha expression on CD4+ T lymphocytes decreases with HIV disease progression and inversely correlates with immune activation. Eur J Immunol **36:**336-44.

132. **Kohler, J. J., D. L. Tuttle, C. R. Coberley, J. W. Sleasman, and M. M. Goodenow.** 2003. Human immunodeficiency virus type 1 (HIV-1) induces activation of multiple STATs in CD4+ cells of lymphocyte or monocyte/macrophage lineages. J Leukoc Biol **73:**407-16.

133. **Kong, X., H. San Juan, A. Behera, M. E. Peeples, J. Wu, R. F. Lockey, and S. S. Mohapatra.** 2004. ERK-1/2 activity is required for efficient RSV infection. FEBS Lett **559:**33-8.

134. **Koopman, G., D. Mortier, S. Hofman, N. Mathy, M. Koutsoukos, P. Ertl, P. Overend, C. van Wely, L. L. Thomsen, B. Wahren, G. Voss, and J. L. Heeney.** 2008. Immune-response profiles induced by human immunodeficiency virus type 1 vaccine DNA, protein or mixed-modality immunization: increased protection from pathogenic simian-human immunodeficiency virus viraemia with protein/DNA combination. J Gen Virol **89:**540-53.

135. **Koopman, G., H. Niphuis, W. Newman, T. Kishimoto, V. Maino, and J. Heeney.** 2001. Decreased expression of IL-2 in central and effector CD4 memory cells during progression to AIDS in rhesus macaques. AIDS **15:**2359-69.

136. **Kornfeld, C., M. J. Ploquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin.** 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. J Clin Invest **115:**1082-91.

137. **Kovacs, J., M. Baseler, R. Dewar, S. Vogel, R. J. Davey, J. Falloon, M. Polis, R. Walker, R. Stevens, and N. Salzman.** 1995. Increases in CD4 T lymphocytes with intermittent courses of interleukin-2 in patients with human immunodeficiency virus infection. A preliminary study. N Engl J Med **332:**567-75.

138. **Kovacs, J., S. Vogel, J. Albert, J. Falloon, R. J. Davey, R. Walker, M. Polis, K. Spooner, J. Metcalf, M. Baseler, G. Fyfe, and H. Lane.** 1996.

Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. N Engl J Med **335:**1350-6.

139. **Kryworuchko, M., V. Pasquier, H. Keller, D. David, C. Goujard, J. Gilquin, J. P. Viard, M. Joussemet, J. F. Delfraissy, and J. Theze.** 2004. Defective interleukin-2-dependent STAT5 signalling in CD8 T lymphocytes from HIV-positive patients: restoration by antiretroviral therapy. AIDS **18:**421-6.

140. **Kuhrt, D.** 2009. Evidence of early B cell dysregulation in SIV infection: Rapid depletion of naïve and memory B cell subsets with delayed reconstitution of the naïve B cell population. Submitted- AIDS Research and Human Retroviruses.

141. **Kuppers, R.** 2008. Human memory B cells: memory B cells of a special kind. Immunol Cell Biol **86:**635-6.

142. **Kuwata, T., H. Dehghani, C. R. Brown, R. Plishka, A. Buckler-White, T. Igarashi, J. Mattapallil, M. Roederer, and V. M. Hirsch.** 2006. Infectious molecular clones from a simian immunodeficiency virus-infected rapid-progressor (RP) macaque: evidence of differential selection of RP-specific envelope mutations in vitro and in vivo. J Virol **80:**1463-75.

143. **Lalezari, J. P., J. A. Beal, P. J. Ruane, C. J. Cohen, E. L. Jacobson, D. Sundin, W. P. Leong, S. P. Raffanti, D. A. Wheeler, R. D. Anderson, P. Keiser, S. R. Schrader, J. C. Goodgame, C. R. Steinhart, R. L. Murphy, M. J. Wolin, and K. A. Smith.** 2000. Low-dose daily subcutaneous interleukin-2 in combination with highly active antiretroviral therapy in HIV+ patients: a randomized controlled trial. HIV Clin Trials **1:**1-15.

144. **Lee, S., C. A. Almeida, M. A. French, and P. Price.** 2004. Persistent HIV-1 replication does not explain low levels of T-cell interferon-gamma mRNA and elevated serum NO(2) (-)/NO(3) (-) in patients with stable CD4 T-cell responses to HAART. Clin Exp Immunol **138:**110-5.

145. **Lee, S. K., and C. D. Surh.** 2005. Role of interleukin-7 in bone and T-cell homeostasis. Immunol Rev **208:**169-80.

146. **Leng, Q., G. Borkow, Z. Weisman, M. Stein, A. Kalinkovich, and Z. Bentwich.** 2001. Immune activation correlates better than HIV plasma viral load with CD4 T-cell decline during HIV infection. J Acquir Immune Defic Syndr **27:**389-97.

147. **Lenz, D. C., S. K. Kurz, E. Lemmens, S. P. Schoenberger, J. Sprent, M. B. Oldstone, and D. Homann.** 2004. IL-7 regulates basal homeostatic proliferation of antiviral CD4+T cell memory. Proc Natl Acad Sci U S A **101:**9357-62.

148. **Leone, A., L. J. Picker, and D. L. Sodora.** 2009. IL-2, IL-7 and IL-15 as immuno-modulators during SIV/HIV vaccination and treatment. Curr HIV Res **7:**83-90.

149. **Levy, Y., C. Lacabaratz, L. Weiss, J. P. Viard, C. Goujard, J. D. Lelievre, F. Boue, J. M. Molina, C. Rouzioux, V. Avettand-Fenoel, T. Croughs, S. Beq, R. Thiebaut, G. Chene, M. Morre, and J. F. Delfraissy.** 2009. Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. J Clin Invest **119:**997-1007.

150. **Li, B., M. J. VanRoey, and K. Jooss.** 2007. Recombinant IL-7 enhances the potency of GM-CSF-secreting tumor cell immunotherapy. Clin Immunol **123:**155-65.

151. **Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase.** 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature **434:**1148-52.

152. **Li, W. Q., Q. Jiang, E. Aleem, P. Kaldis, A. R. Khaled, and S. K. Durum.** 2006. IL-7 promotes T cell proliferation through destabilization of p27Kip1. J Exp Med **203:**573-82.

153. **Lifson, A., F. Rhame, W. Belloso, U. Dragsted, W. El-Sadr, J. Gatell, J. Hoy, E. Krum, R. Nelson, C. Pedersen, S. Pett, and R. J. Davey.** 2006. Reporting and evaluation of HIV-related clinical endpoints in two multicenter international clinical trials. HIV Clin Trials **7:**125-41.

154. **Lin, Y. L., P. Portales, M. Segondy, V. Baillat, C. M. de Boever, V. Le Moing, B. Reant, B. Montes, J. Clot, J. Reynes, and P. Corbeau.** 2005. CXCR4 overexpression during the course of HIV-1 infection correlates with the emergence of X4 strains. J Acquir Immune Defic Syndr **39:**530-6.

155. **Liu, Z., W. Cumberland, L. Hultin, H. Prince, R. Detels, and J. Giorgi.** 1997. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol **16:**83-92.

156. **Llano, A., J. Barretina, A. Gutierrez, J. Blanco, C. Cabrera, B. Clotet, and J. A. Este.** 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:**10319-25.

157. **Lodolce, J., P. Burkett, R. Koka, D. Boone, M. Chien, F. Chan, M. Madonia, S. Chai, and A. Ma.** 2002. Interleukin-15 and the regulation of lymphoid homeostasis. Mol Immunol **39:**537-44.

158. **Lodolce, J., P. Burkett, R. Koka, D. Boone, and A. Ma.** 2002. Regulation of lymphoid homeostasis by interleukin-15. Cytokine Growth Factor Rev **13:**429-39.

159. **Long, S., and J. Buckner.** 2008. Combination of rapamycin and IL-2 increases de novo induction of human CD4(+)CD25(+)FOXP3(+) T cells. J Autoimmun **30:**293-302.

160. **Lu, H., Z. Zhao, T. Kalina, T. Gillespy, 3rd, D. Liggitt, R. G. Andrews, D. G. Maloney, H. P. Kiem, and J. Storek.** 2005. Interleukin-7 improves reconstitution of antiviral CD4 T cells. Clin Immunol **114:**30-41.

161. **Ma, A., R. Koka, and P. Burkett.** 2006. Diverse functions of IL-2, IL-15, and IL-7 in lymphoid homeostasis. Annu Rev Immunol **24:**657-79.

162. **MacPherson, P. A., C. Fex, J. Sanchez-Dardon, N. Hawley-Foss, and J. B. Angel.** 2001. Interleukin-7 receptor expression on CD8(+) T cells is reduced in HIV infection and partially restored with effective antiretroviral therapy. J Acquir Immune Defic Syndr **28:**454-7.

163. **Maki, K., S. Sunaga, Y. Komagata, Y. Kodaira, A. Mabuchi, H. Karasuyama, K. Yokomuro, J. I. Miyazaki, and K. Ikuta.** 1996. Interleukin 7 receptor-deficient mice lack gammadelta T cells. Proc Natl Acad Sci U S A **93:**7172-7.

164. **Malaspina, A., S. Moir, S. Kottilil, C. W. Hallahan, L. A. Ehler, S. Liu, M. A. Planta, T. W. Chun, and A. S. Fauci.** 2003. Deleterious effect of HIV-1 plasma viremia on B cell costimulatory function. J Immunol **170:**5965-72.

165. **Managlia, E., A. Landay, and L. Al-Harthi.** 2006. Interleukin-7 induces HIV replication in primary naive T cells through a nuclear factor of activated T cell (NFAT)-dependent pathway. Virology **350:**443-52.

166. **Marchetti, G., F. Franzetti, and A. Gori.** 2005. Partial immune reconstitution following highly active antiretroviral therapy: can adjuvant interleukin-2 fill the gap? J Antimicrob Chemother **55:**401-9.

167. **Marziali, M., W. De Santis, R. Carello, W. Leti, A. Esposito, A. Isgrò, C. Fimiani, M. Sirianni, I. Mezzaroma, and F. Aiuti.** 2006. T-cell homeostasis alteration in HIV-1 infected subjects with low CD4 T-cell count despite undetectable virus load during HAART. AIDS **20:**2033-41.

168. **Mathiot, N. D., R. Krueger, M. A. French, and P. Price.** 2001. Percentage of CD3+CD4-CD8-gammadeltaTCR- T cells is increased HIV disease. AIDS Res Hum Retroviruses **17:**977-80.

169. **Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer.** 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature **434:**1093-7.

170. **Meazza, R., S. Basso, A. Gaggero, D. Detotero, L. Trentin, R. Pereno, B. Azzarone, and S. Ferrini.** 1998. Interleukin (IL)-15 induces survival

and proliferation of the growth factor-dependent acute myeloid leukemia M-07e through the IL-2 receptor beta/gamma. Int J Cancer **78:**189-95.

171. **Melchionda, F., T. J. Fry, M. J. Milliron, M. A. McKirdy, Y. Tagaya, and C. L. Mackall.** 2005. Adjuvant IL-7 or IL-15 overcomes immunodominance and improves survival of the CD8+ memory cell pool. J Clin Invest **115:**1177-87.

172. **Mercier, F., M. R. Boulassel, B. Yassine-Diab, C. Tremblay, N. F. Bernard, R. P. Sekaly, and J. P. Routy.** 2008. Persistent human immunodeficiency virus-1 antigenaemia affects the expression of interleukin-7Ralpha on central and effector memory CD4+ and CD8+ T cell subsets. Clin Exp Immunol **152:**72-80.

173. **Miller, E. D., J. A. Smith, M. Lichtinger, L. Wang, and L. Su.** 2003. Activation of the signal transducer and activator of transcription 1 signaling pathway in thymocytes from HIV-1-infected human thymus. AIDS **17:**1269-77.

174. **Milne, C. D., H. E. Fleming, Y. Zhang, and C. J. Paige.** 2004. Mechanisms of selection mediated by interleukin-7, the preBCR, and hemokinin-1 during B-cell development. Immunol Rev **197:**75-88.

175. **Minigo, G., T. Woodberry, K. A. Piera, E. Salwati, E. Tjitra, E. Kenangalem, R. N. Price, C. R. Engwerda, N. M. Anstey, and M. Plebanski.** 2009. Parasite-dependent expansion of TNF receptor II-positive regulatory T cells with enhanced suppressive activity in adults with severe malaria. PLoS Pathog **5:**e1000402.

176. **Miranda-Carús, M., M. Benito-Miguel, M. Llamas, A. Balsa, and E. Martín-Mola.** 2005. Human T cells constitutively express IL-15 that promotes ex vivo T cell homeostatic proliferation through autocrine/juxtacrine loops. J Immunol **175:**3656-62.

177. **Mitsuyasu, R., R. Gelman, D. W. Cherng, A. Landay, J. Fahey, R. Reichman, A. Erice, R. P. Bucy, J. M. Kilby, M. M. Lederman, C. D. Hamilton, J. Lertora, B. L. White, P. Tebas, A. M. Duliege, and R. B. Pollard.** 2007. The virologic, immunologic, and clinical effects of interleukin 2 with potent antiretroviral therapy in patients with moderately

advanced human immunodeficiency virus infection: a randomized controlled clinical trial--AIDS Clinical Trials Group 328. Arch Intern Med **167:**597-605.

178. **Moir, S., and A. S. Fauci.** 2009. B cells in HIV infection and disease. Nat Rev Immunol **9:**235-45.

179. **Moir, S., J. Ho, A. Malaspina, W. Wang, A. C. DiPoto, M. A. O'Shea, G. Roby, S. Kottilil, J. Arthos, M. A. Proschan, T. W. Chun, and A. S. Fauci.** 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med **205:**1797-805.

180. **Moir, S., A. Malaspina, K. M. Ogwaro, E. T. Donoghue, C. W. Hallahan, L. A. Ehler, S. Liu, J. Adelsberger, R. Lapointe, P. Hwu, M. Baseler, J. M. Orenstein, T. W. Chun, J. A. Mican, and A. S. Fauci.** 2001. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. Proc Natl Acad Sci U S A **98:**10362-7.

181. **Moir, S., A. Malaspina, O. K. Pickeral, E. T. Donoghue, J. Vasquez, N. J. Miller, S. R. Krishnan, M. A. Planta, J. F. Turney, J. S. Justement, S. Kottilil, M. Dybul, J. M. Mican, C. Kovacs, T. W. Chun, C. E. Birse, and A. S. Fauci.** 2004. Decreased survival of B cells of HIV-viremic patients mediated by altered expression of receptors of the TNF superfamily. J Exp Med **200:**587-99.

182. **Monceaux, V., J. Estaquier, M. Fevrier, M. C. Cumont, Y. Riviere, A. M. Aubertin, J. C. Ameisen, and B. Hurtrel.** 2003. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. AIDS **17:**1585-96.

183. **Moniuszko, M., Y. Edghill-Smith, D. Venzon, L. Stevceva, J. Nacsa, E. Tryniszewska, W. P. Tsai, and G. Franchini.** 2006. Decreased number of CD4+ and CD8+ T cells that express the interleukin-7 receptor in blood and tissues of SIV-infected macaques. Virology **356:**188-97.

184. **Moniuszko, M., T. Fry, W. P. Tsai, M. Morre, B. Assouline, P. Cortez, M. G. Lewis, S. Cairns, C. Mackall, and G. Franchini.** 2004.

Recombinant interleukin-7 induces proliferation of naive macaque CD4+ and CD8+ T cells in vivo. J Virol **78:**9740-9.

185. **Montoya, C. J., M. F. Toro, C. Aguirre, A. Bustamante, M. Hernandez, L. P. Arango, M. Echeverry, A. E. Arango, M. C. Prada, P. Alarcon Hdel, and M. Rojas.** 2007. Abnormal humoral immune response to influenza vaccination in pediatric type-1 human immunodeficiency virus infected patients receiving highly active antiretroviral therapy. Mem Inst Oswaldo Cruz **102:**501-8.

186. **Morrow, M., A. Valentin, R. Little, R. Yarchoan, and G. N. Pavlakis.** 2008. A splenic marginal zone-like peripheral blood CD27+B220- B cell population is preferentially depleted in HIV type 1-infected individuals. AIDS Res Hum Retroviruses **24:**621-33.

187. **Mortier, E., T. Woo, R. Advincula, S. Gozalo, and A. Ma.** 2008. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. J Exp Med **205:**1213-25.

188. **Mueller, Y., D. Do, S. Altork, C. Artlett, E. Gracely, C. Katsetos, A. Legido, F. Villinger, J. Altman, C. Brown, M. Lewis, and P. Katsikis.** 2008. IL-15 treatment during acute simian immunodeficiency virus (SIV) infection increases viral set point and accelerates disease progression despite the induction of stronger SIV-specific CD8+ T cell responses. J Immunol **180:**350-60.

189. **Mueller, Y., C. Petrovas, P. Bojczuk, I. Dimitriou, B. Beer, P. Silvera, F. Villinger, J. Cairns, E. Gracely, M. Lewis, and P. Katsikis.** 2005. Interleukin-15 increases effector memory CD8+ t cells and NK Cells in simian immunodeficiency virus-infected macaques. J Virol **79:**4877-85.

190. **Musso, T., L. Calosso, M. Zucca, M. Millesimo, D. Ravarino, M. Giovarelli, F. Malavasi, A. Ponzi, R. Paus, and S. Bulfone-Paus.** 1999. Human monocytes constitutively express membrane-bound, biologically active, and interferon-gamma-upregulated interleukin-15. Blood **93:**3531-9.

191. **Muthukumar, A., A. Wozniakowski, M. C. Gauduin, M. Paiardini, H. M. McClure, R. P. Johnson, G. Silvestri, and D. L. Sodora.** 2004.

Elevated interleukin-7 levels not sufficient to maintain T-cell homeostasis during simian immunodeficiency virus-induced disease progression. Blood **103:**973-9.

192. **Muthukumar, A., D. Zhou, M. Paiardini, A. Barry, K. Cole, H. McClure, S. Staprans, G. Silvestri, and D. Sodora.** 2005. Timely triggering of homeostatic mechanisms involved in the regulation of T-cell levels in SIVsm-infected sooty mangabeys. Blood **106:**3839-45.

193. **Nacsa, J., Y. Edghill-Smith, W. Tsai, D. Venzon, E. Tryniszewska, A. Hryniewicz, M. Moniuszko, A. Kinter, K. Smith, and G. Franchini.** 2005. Contrasting effects of low-dose IL-2 on vaccine-boosted simian immunodeficiency virus (SIV)-specific CD4+ and CD8+ T cells in macaques chronically infected with SIVmac251. J Immunol **174:**1913-21.

194. **Nanjappa, S. G., J. H. Walent, M. Morre, and M. Suresh.** 2008. Effects of IL-7 on memory CD8 T cell homeostasis are influenced by the timing of therapy in mice. J Clin Invest **118:**1027-39.

195. **Napolitano, L.** 2003. Approaches to immune reconstitution in HIV infection. Top HIV Med **11:**160-3.

196. **Napolitano, L., T. Burt, P. Bacchetti, Y. Barrón, A. French, A. Kovacs, K. Anastos, M. Young, J. McCune, and R. Greenblatt.** 2005. Increased circulating interleukin-7 levels in HIV-1-infected women. J Acquir Immune Defic Syndr **40:**581-4.

197. **Napolitano, L., R. Grant, S. Deeks, D. Schmidt, S. De Rosa, L. Herzenberg, B. Herndier, J. Andersson, and J. McCune.** 2001. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. Nat Med **7:**73-9.

198. **Napolitano, L. A., C. A. Stoddart, M. B. Hanley, E. Wieder, and J. M. McCune.** 2003. Effects of IL-7 on early human thymocyte progenitor cells in vitro and in SCID-hu Thy/Liv mice. J Immunol **171:**645-54.

199. **Ndolo, T., N. K. Dhillon, H. Nguyen, M. Guadalupe, M. Mudryj, and S. Dandekar.** 2002. Simian immunodeficiency virus Nef protein delays

the progression of CD4+ T cells through G1/S phase of the cell cycle. J Virol **76:**3587-95.

200. **Nemes, E., E. Lugli, M. Nasi, R. Ferraresi, M. Pinti, R. Bugarini, V. Borghi, F. Prati, R. Esposito, A. Cossarizza, and C. Mussini.** 2006. Immunophenotype of HIV+ patients during CD4 cell-monitored treatment interruption: role of the IL-7/IL-7 receptor system. AIDS **20:**2021-32.

201. **Nerrienet, E., M. L. Santiago, Y. Foupouapouognigni, E. Bailes, N. I. Mundy, B. Njinku, A. Kfutwah, M. C. Muller-Trutwin, F. Barre-Sinoussi, G. M. Shaw, P. M. Sharp, B. H. Hahn, and A. Ayouba.** 2005. Simian immunodeficiency virus infection in wild-caught chimpanzees from cameroon. J Virol **79:**1312-9.

202. **Nishimura, Y., T. Igarashi, O. K. Donau, A. Buckler-White, C. Buckler, B. A. Lafont, R. M. Goeken, S. Goldstein, V. M. Hirsch, and M. A. Martin.** 2004. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc Natl Acad Sci U S A **101:**12324-9.

203. **Northrup, D. L., and D. Allman.** 2008. Transcriptional regulation of early B cell development. Immunol Res **42:**106-17.

204. **Nugeyre, M., V. Monceaux, S. Beq, M. Cumont, R. Ho Tsong Fang, L. Chêne, M. Morre, F. Barré-Sinoussi, B. Hurtrel, and N. Israël.** 2003. IL-7 stimulates T cell renewal without increasing viral replication in simian immunodeficiency virus-infected macaques. J Immunol **171:**4447-53.

205. **Nunn, M. F., and J. W. Marsh.** 1996. Human immunodeficiency virus type 1 Nef associates with a member of the p21-activated kinase family. J Virol **70:**6157-61.

206. **Nunnari, G., and R. J. Pomerantz.** 2005. IL-7 as a potential therapy for HIV-1-infected individuals. Expert Opin Biol Ther **5:**1421-6.

207. **Okkenhaug, K., and B. Vanhaesebroeck.** 2003. PI3K in lymphocyte development, differentiation and activation. Nat Rev Immunol **3:**317-30.

208. **Okoye, A., M. Meier-Schellersheim, J. M. Brenchley, S. I. Hagen, J. M. Walker, M. Rohankhedkar, R. Lum, J. B. Edgar, S. L. Planer, A. Legasse, A. W. Sylwester, M. Piatak, Jr., J. D. Lifson, V. C. Maino, D. L. Sodora, D. C. Douek, M. K. Axthelm, Z. Grossman, and L. J. Picker.** 2007. Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection. J Exp Med **204:**2171-85.

209. **Orandle, M. S., K. C. Williams, A. G. MacLean, S. V. Westmoreland, and A. A. Lackner.** 2001. Macaques with rapid disease progression and simian immunodeficiency virus encephalitis have a unique cytokine profile in peripheral lymphoid tissues. J Virol **75:**4448-52.

210. **Ostrowski, M., T. Chun, S. Justement, I. Motola, M. Spinelli, J. Adelsberger, L. Ehler, S. Mizell, C. Hallahan, and A. Fauci.** 1999. Both memory and CD45RA+/CD62L+ naive CD4(+) T cells are infected in human immunodeficiency virus type 1-infected individuals. J Virol **73:**6430-5.

211. **Pahwa, R., T. W. McCloskey, O. C. Aroniadis, N. Strbo, S. Krishnan, and S. Pahwa.** 2006. CD8+ T cells in HIV disease exhibit cytokine receptor perturbation and poor T cell receptor activation but are responsive to gamma-chain cytokine-driven proliferation. J Infect Dis **193:**879-87.

212. **Paiardini, M., B. Cervasi, H. Albrecht, A. Muthukumar, R. Dunham, S. Gordon, H. Radziewicz, G. Piedimonte, M. Magnani, M. Montroni, S. M. Kaech, A. Weintrob, J. D. Altman, D. L. Sodora, M. B. Feinberg, and G. Silvestri.** 2005. Loss of CD127 expression defines an expansion of effector CD8+ T cells in HIV-infected individuals. J Immunol **174:**2900-9.

213. **Paiardini, M., B. Cervasi, B. Sumpter, H. M. McClure, D. L. Sodora, M. Magnani, S. I. Staprans, G. Piedimonte, and G. Silvestri.** 2006. Perturbations of cell cycle control in T cells contribute to the different outcomes of simian immunodeficiency virus infection in rhesus macaques and sooty mangabeys. J Virol **80:**634-42.

214. **Park, J. I., C. J. Strock, D. W. Ball, and B. D. Nelkin.** 2003. The Ras/Raf/MEK/extracellular signal-regulated kinase pathway induces

autocrine-paracrine growth inhibition via the leukemia inhibitory factor/JAK/STAT pathway. Mol Cell Biol **23:**543-54.

215. **Parrish, Y. K., I. Baez, T. A. Milford, A. Benitez, N. Galloway, J. W. Rogerio, E. Sahakian, M. Kagoda, G. Huang, Q. L. Hao, Y. Sevilla, L. W. Barsky, E. Zielinska, M. A. Price, N. R. Wall, S. Dovat, and K. J. Payne.** 2009. IL-7 Dependence in human B lymphopoiesis increases during progression of ontogeny from cord blood to bone marrow. J Immunol **182:**4255-66.

216. **Pericle, F., L. A. Pinto, S. Hicks, R. A. Kirken, G. Sconocchia, J. Rusnak, M. J. Dolan, G. M. Shearer, and D. M. Segal.** 1998. HIV-1 infection induces a selective reduction in STAT5 protein expression. J Immunol **160:**28-31.

217. **Peruzzi, M., C. Azzari, L. Galli, A. Vierucci, and M. De Martino.** 2002. Highly active antiretroviral therapy restores in vitro mitogen and antigen-specific T-lymphocyte responses in HIV-1 perinatally infected children despite virological failure. Clin Exp Immunol **128:**365-71.

218. **Peschon, J. J., P. J. Morrissey, K. H. Grabstein, F. J. Ramsdell, E. Maraskovsky, B. C. Gliniak, L. S. Park, S. F. Ziegler, D. E. Williams, C. B. Ware, J. D. Meyer, and B. L. Davison.** 1994. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med **180:**1955-60.

219. **Picker, L., S. Hagen, R. Lum, E. Reed-Inderbitzin, L. Daly, A. Sylwester, J. Walker, D. Siess, M. J. Piatak, C. Wang, D. Allison, V. Maino, J. Lifson, T. Kodama, and M. Axthelm.** 2004. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. J Exp Med **200:**1299-314.

220. **Picker, L., E. Reed-Inderbitzin, S. Hagen, J. Edgar, S. Hansen, A. Legasse, S. Planer, M. J. Piatak, J. Lifson, V. Maino, M. Axthelm, and F. Villinger.** 2006. IL-15 induces CD4 effector memory T cell production and tissue emigration in nonhuman primates. J Clin Invest **116:**1514-24.

221. **Pitcher, C. J., S. I. Hagen, J. M. Walker, R. Lum, B. L. Mitchell, V. C. Maino, M. K. Axthelm, and L. J. Picker.** 2002. Development and homeostasis of T cell memory in rhesus macaque. J Immunol **168:**29-43.

222. **Pleschka, S., T. Wolff, C. Ehrhardt, G. Hobom, O. Planz, U. R. Rapp, and S. Ludwig.** 2001. Influenza virus propagation is impaired by inhibition of the Raf/MEK/ERK signalling cascade. Nat Cell Biol **3:**301-5.

223. **Popik, W., and P. M. Pitha.** 1998. Early activation of mitogen-activated protein kinase kinase, extracellular signal-regulated kinase, p38 mitogen-activated protein kinase, and c-Jun N-terminal kinase in response to binding of simian immunodeficiency virus to Jurkat T cells expressing CCR5 receptor. Virology **252:**210-7.

224. **Popik, W., and P. M. Pitha.** 2000. Inhibition of CD3/CD28-mediated activation of the MEK/ERK signaling pathway represses replication of X4 but not R5 human immunodeficiency virus type 1 in peripheral blood CD4(+) T lymphocytes. J Virol **74:**2558-66.

225. **Potter, S. J., C. Lacabaratz, O. Lambotte, S. Perez-Patrigeon, B. Vingert, M. Sinet, J. H. Colle, A. Urrutia, D. Scott-Algara, F. Boufassa, J. F. Delfraissy, J. Theze, A. Venet, and L. A. Chakrabarti.** 2007. Preserved central memory and activated effector memory CD4+ T-cell subsets in human immunodeficiency virus controllers: an ANRS EP36 study. J Virol **81:**13904-15.

226. **Puel, A., and W. J. Leonard.** 2000. Mutations in the gene for the IL-7 receptor result in T(-)B(+)NK(+) severe combined immunodeficiency disease. Curr Opin Immunol **12:**468-73.

227. **Puel, A., S. F. Ziegler, R. H. Buckley, and W. J. Leonard.** 1998. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. Nat Genet **20:**394-7.

228. **Rajnavolgyi, E., N. Benbernou, B. Rethi, D. Reynolds, H. A. Young, M. Magocsi, K. Muegge, and S. K. Durum.** 2002. IL-7 withdrawal induces a stress pathway activating p38 and Jun N-terminal kinases. Cell Signal **14:**761-9.

229. **Rappl, G., A. Kapsokefalou, C. Heuser, M. Rössler, S. Ugurel, W. Tilgen, U. Reinhold, and H. Abken.** 2001. Dermal fibroblasts sustain proliferation of activated T cells via membrane-bound interleukin-15 upon long-term stimulation with tumor necrosis factor-alpha. J Invest Dermatol **116:**102-9.

230. **Read, S. W., J. Higgins, J. A. Metcalf, R. A. Stevens, A. Rupert, M. C. Nason, H. C. Lane, and I. Sereti.** 2006. Decreased CD127 expression on T Cells in HIV-1-infected adults receiving antiretroviral therapy with or without intermittent IL-2 therapy. J Acquir Immune Defic Syndr **42:**537-44.

231. **Rerks-Ngarm, S., P. Pitisuttithum, S. Nitayaphan, J. Kaewkungwal, J. Chiu, R. Paris, N. Premsri, C. Namwat, M. de Souza, E. Adams, M. Benenson, S. Gurunathan, J. Tartaglia, J. G. McNeil, D. P. Francis, D. Stablein, D. L. Birx, S. Chunsuttiwat, C. Khamboonruang, P. Thongcharoen, M. L. Robb, N. L. Michael, P. Kunasol, and J. H. Kim.** 2009. Vaccination with ALVAC and AIDSVAX to Prevent HIV-1 Infection in Thailand. N Engl J Med.

232. **Resino, S., I. Galán, R. Correa, L. Pajuelo, J. Bellón, and M. Muñoz-Fernández.** 2005. Homeostatic role of IL-7 in HIV-1 infected children on HAART: association with immunological and virological parameters. Acta Paediatr **94:**170-7.

233. **Rethi, B., C. Fluur, A. Atlas, M. Krzyzowska, F. Mowafi, S. Grutzmeier, A. De Milito, R. Bellocco, K. I. Falk, E. Rajnavolgyi, and F. Chiodi.** 2005. Loss of IL-7Ralpha is associated with CD4 T-cell depletion, high interleukin-7 levels and CD28 down-regulation in HIV infected patients. AIDS **19:**2077-86.

234. **Ribeiro, R. M.** 2007. Dynamics of CD4+ T cells in HIV-1 infection. Immunol Cell Biol **85:**287-94.

235. **Riou, C., B. Yassine-Diab, J. Van grevenynghe, R. Somogyi, L. D. Greller, D. Gagnon, S. Gimmig, P. Wilkinson, Y. Shi, M. J. Cameron, R. Campos-Gonzalez, R. S. Balderas, D. Kelvin, R. P. Sekaly, and E. K. Haddad.** 2007. Convergence of TCR and cytokine signaling leads to

FOXO3a phosphorylation and drives the survival of CD4+ central memory T cells. J Exp Med **204:**79-91.

236. **Roifman, C. M., J. Zhang, D. Chitayat, and N. Sharfe.** 2000. A partial deficiency of interleukin-7R alpha is sufficient to abrogate T-cell development and cause severe combined immunodeficiency. Blood **96:**2803-7.

237. **Rosenberg, S. A., C. Sportes, M. Ahmadzadeh, T. J. Fry, L. T. Ngo, S. L. Schwarz, M. Stetler-Stevenson, K. E. Morton, S. A. Mavroukakis, M. Morre, R. Buffet, C. L. Mackall, and R. E. Gress.** 2006. IL-7 administration to humans leads to expansion of CD8+ and CD4+ cells but a relative decrease of CD4+ T-regulatory cells. J Immunother **29:**313-9.

238. **Rosenzweig, M., M. A. DeMaria, D. M. Harper, S. Friedrich, R. K. Jain, and R. P. Johnson.** 1998. Increased rates of CD4(+) and CD8(+) T lymphocyte turnover in simian immunodeficiency virus-infected macaques. Proc Natl Acad Sci U S A **95:**6388-93.

239. **Rückert, R., K. Asadullah, M. Seifert, V. Budagian, R. Arnold, C. Trombotto, R. Paus, and S. Bulfone-Paus.** 2000. Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? J Immunol **165:**2240-50.

240. **Sandau, M., C. Winstead, and S. Jameson.** 2007. IL-15 is required for sustained lymphopenia-driven proliferation and accumulation of CD8 T cells. J Immunol **179:**120-5.

241. **Sasaki, S., M. C. Jaimes, T. H. Holmes, C. L. Dekker, K. Mahmood, G. W. Kemble, A. M. Arvin, and H. B. Greenberg.** 2007. Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. J Virol **81:**215-28.

242. **Sasson, S. C., J. J. Zaunders, G. Zanetti, E. M. King, K. M. Merlin, D. E. Smith, K. K. Stanley, D. A. Cooper, and A. D. Kelleher.** 2006. Increased plasma interleukin-7 level correlates with decreased CD127 and Increased CD132 extracellular expression on T cell subsets in patients with HIV-1 infection. J Infect Dis **193:**505-14.

243. **Satoh, J., K. Kurohara, M. Yukitake, and Y. Kuroda.** 1998. Interleukin-15, a T-cell growth factor, is expressed in human neural cell lines and tissues. J Neurol Sci **155:**170-7.

244. **Schacker, T., J. Brenchley, G. Beilman, C. Reilly, S. Pambuccian, J. Taylor, D. Skarda, M. Larson, D. Douek, and A. Haase.** 2006. Lymphatic tissue fibrosis is associated with reduced numbers of naive CD4+ T cells in human immunodeficiency virus type 1 infection. Clin Vaccine Immunol **13:**556-60.

245. **Schluns, K., W. Kieper, S. Jameson, and L. Lefrançois.** 2000. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. Nat Immunol **1:**426-32.

246. **Schluns, K., and L. Lefrançois.** 2003. Cytokine control of memory T-cell development and survival. Nat Rev Immunol **3:**269-79.

247. **Schmitt, N., L. Chene, D. Boutolleau, M. T. Nugeyre, E. Guillemard, P. Versmisse, C. Jacquemot, F. Barre-Sinoussi, and N. Israel.** 2003. Positive regulation of CXCR4 expression and signaling by interleukin-7 in CD4+ mature thymocytes correlates with their capacity to favor human immunodeficiency X4 virus replication. J Virol **77:**5784-93.

248. **Scripture-Adams, D. D., D. G. Brooks, Y. D. Korin, and J. A. Zack.** 2002. Interleukin-7 induces expression of latent human immunodeficiency virus type 1 with minimal effects on T-cell phenotype. J Virol **76:**13077-82.

249. **Seki, Y., J. Yang, M. Okamoto, S. Tanaka, R. Goitsuka, M. A. Farrar, and M. Kubo.** 2007. IL-7/STAT5 cytokine signaling pathway is essential but insufficient for maintenance of naive CD4 T cell survival in peripheral lymphoid organs. J Immunol **178:**262-70.

250. **Selliah, N., and T. H. Finkel.** 1998. Cutting edge: JAK3 activation and rescue of T cells from HIV gp120-induced unresponsiveness. J Immunol **160:**5697-701.

251. **Sereti, I., R. M. Dunham, J. Spritzler, E. Aga, M. A. Proschan, K. Medvik, C. A. Battaglia, A. L. Landay, S. Pahwa, M. A. Fischl, D. M.**

Asmuth, A. R. Tenorio, J. D. Altman, L. Fox, S. Moir, A. Malaspina, M. Morre, R. Buffet, G. Silvestri, and M. M. Lederman. 2009. IL-7 administration drives T cell cycle entry and expansion in HIV-1 infection. Blood.

252. **Sereti, I., H. Martinez-Wilson, J. Metcalf, M. Baseler, C. Hallahan, B. Hahn, R. Hengel, R. Davey, J. Kovacs, and H. Lane.** 2002. Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of CD4(+)/CD25(+) T cells. Blood **100:**2159-67.

253. **Sharma, T. S., J. Hughes, A. Murillo, J. Riley, A. Soares, F. Little, C. D. Mitchell, and W. A. Hanekom.** 2008. CD8+ T-cell interleukin-7 receptor alpha expression as a potential indicator of disease status in HIV-infected children. PLoS One **3:**e3986.

254. **Sharma-Walia, N., H. H. Krishnan, P. P. Naranatt, L. Zeng, M. S. Smith, and B. Chandran.** 2005. ERK1/2 and MEK1/2 induced by Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) early during infection of target cells are essential for expression of viral genes and for establishment of infection. J Virol **79:**10308-29.

255. **Shelton, J. G., L. S. Steelman, J. T. Lee, S. L. Knapp, W. L. Blalock, P. W. Moye, R. A. Franklin, S. C. Pohnert, A. M. Mirza, M. McMahon, and J. A. McCubrey.** 2003. Effects of the RAF/MEK/ERK and PI3K/AKT signal transduction pathways on the abrogation of cytokine-dependence and prevention of apoptosis in hematopoietic cells. Oncogene **22:**2478-92.

256. **Shinozaki, M., J. Hirahashi, T. Lebedeva, F. Liew, D. Salant, R. Maron, and V. Kelley.** 2002. IL-15, a survival factor for kidney epithelial cells, counteracts apoptosis and inflammation during nephritis. J Clin Invest **109:**951-60.

257. **Sierra, S., B. Kupfer, and R. Kaiser.** 2005. Basics of the virology of HIV-1 and its replication. J Clin Virol **34:**233-44.

258. **Silvestri, G., A. Fedanov, S. Germon, N. Kozyr, W. Kaiser, D. Garber, H. McClure, M. Feinberg, and S. Staprans.** 2005. Divergent host

responses during primary simian immunodeficiency virus SIVsm infection of natural sooty mangabey and nonnatural rhesus macaque hosts. J Virol **79:**4043-54.

259. **Sirskyj, D., J. Theze, A. Kumar, and M. Kryworuchko.** 2008. Disruption of the gamma c cytokine network in T cells during HIV infection. Cytokine **43:**1-14.

260. **Smithgall, M., J. Wong, K. Critchett, and O. Haffar.** 1996. IL-7 up-regulates HIV-1 replication in naturally infected peripheral blood mononuclear cells. J Immunol **156:**2324-30.

261. **Smyth, C. M., S. L. Ginn, C. T. Deakin, G. J. Logan, and I. E. Alexander.** 2007. Limiting {gamma}c expression differentially affects signaling via the interleukin (IL)-7 and IL-15 receptors. Blood **110:**91-8.

262. **Snyder, K., C. Mackall, and T. Fry.** 2006. IL-7 in allogeneic transplant: clinical promise and potential pitfalls. Leuk Lymphoma **47:**1222-8.

263. **Song, H., E. Nakayama, and T. Shioda.** 2006. Effects of human interleukin 7 on HIV-1 replication in monocyte-derived human macrophages. AIDS **20:**937-9.

264. **Sontag, E., S. Fedorov, C. Kamibayashi, D. Robbins, M. Cobb, and M. Mumby.** 1993. The interaction of SV40 small tumor antigen with protein phosphatase 2A stimulates the map kinase pathway and induces cell proliferation. Cell **75:**887-97.

265. **Sparger, E. E., R. A. Dubie, B. L. Shacklett, K. S. Cole, W. L. Chang, and P. A. Luciw.** 2008. Vaccination of rhesus macaques with a vif-deleted simian immunodeficiency virus proviral DNA vaccine. Virology **374:**261-72.

266. **Sportès, C., F. Hakim, S. Memon, H. Zhang, K. Chua, M. Brown, T. Fleisher, M. Krumlauf, R. Babb, C. Chow, T. Fry, J. Engels, R. Buffet, M. Morre, R. Amato, D. Venzon, R. Korngold, A. Pecora, R. Gress, and C. Mackall.** 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:**1701-14.

267. **Stahl-Hennig, C., M. Eisenblatter, E. Jasny, T. Rzehak, K. Tenner-Racz, C. Trumpfheller, A. M. Salazar, K. Uberla, K. Nieto, J. Kleinschmidt, R. Schulte, L. Gissmann, M. Muller, A. Sacher, P. Racz, R. M. Steinman, M. Uguccioni, and R. Ignatius.** 2009. Synthetic double-stranded RNAs are adjuvants for the induction of T helper 1 and humoral immune responses to human papillomavirus in rhesus macaques. PLoS Pathog **5:**e1000373.

268. **Stevens, A., J. Matthews, P. Andres, V. Baffis, X. Zheng, D. Chae, J. Smith, T. Strom, and W. Maslinski.** 1997. Interleukin-15 signals T84 colonic epithelial cells in the absence of the interleukin-2 receptor beta-chain. Am J Physiol **272:**G1201-8.

269. **Subramaniam, K. S., R. Segal, R. H. Lyles, M. C. Rodriguez-Barradas, and L. A. Pirofski.** 2003. Qualitative change in antibody responses of human immunodeficiency virus-infected individuals to pneumococcal capsular polysaccharide vaccination associated with highly active antiretroviral therapy. J Infect Dis **187:**758-68.

270. **Sun, J. C., S. M. Lehar, and M. J. Bevan.** 2006. Augmented IL-7 signaling during viral infection drives greater expansion of effector T cells but does not enhance memory. J Immunol **177:**4458-63.

271. **Sun, J. C., M. A. Williams, and M. J. Bevan.** 2004. CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. Nat Immunol **5:**927-33.

272. **Swainson, L., S. Kinet, C. Mongellaz, M. Sourisseau, T. Henriques, and N. Taylor.** 2007. IL-7-induced proliferation of recent thymic emigrants requires activation of the PI3K pathway. Blood **109:**1034-42.

273. **Taguchi, T., H. Takenouchi, Y. Shiozawa, J. Matsui, N. Kitamura, Y. Miyagawa, Y. U. Katagiri, T. Takahashi, H. Okita, J. Fujimoto, and N. Kiyokawa.** 2007. Interleukin-7 contributes to human pro-B-cell development in a mouse stromal cell-dependent culture system. Exp Hematol **35:**1398-407.

274. **Takehisa, J., M. H. Kraus, A. Ayouba, E. Bailes, F. Van Heuverswyn, J. M. Decker, Y. Li, R. S. Rudicell, G. H. Learn, C. Neel, E. M. Ngole,**

G. M. Shaw, M. Peeters, P. M. Sharp, and B. H. Hahn. 2009. Origin and biology of simian immunodeficiency virus in wild-living western gorillas. J Virol **83:**1635-48.

275. **Tan, J. T., E. Dudl, E. LeRoy, R. Murray, J. Sprent, K. I. Weinberg, and C. D. Surh.** 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. Proc Natl Acad Sci U S A **98:**8732-7.

276. **Thomson, C. W., B. P. Lee, and L. Zhang.** 2006. Double-negative regulatory T cells: non-conventional regulators. Immunol Res **35:**163-78.

277. **Tilton, B., L. Ho, E. Oberlin, P. Loetscher, F. Baleux, I. Clark-Lewis, and M. Thelen.** 2000. Signal transduction by CXC chemokine receptor 4. Stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes. J Exp Med **192:**313-24.

278. **Titanji, K., A. De Milito, A. Cagigi, R. Thorstensson, S. Grutzmeier, A. Atlas, B. Hejdeman, F. P. Kroon, L. Lopalco, A. Nilsson, and F. Chiodi.** 2006. Loss of memory B cells impairs maintenance of long-term serologic memory during HIV-1 infection. Blood **108:**1580-7.

279. **Turka, L., and P. Walsh.** 2008. IL-2 signaling and CD4+ CD25+ Foxp3+ regulatory T cells. Front Biosci **13:**1440-6.

280. **van Grevenynghe, J., F. A. Procopio, Z. He, N. Chomont, C. Riou, Y. Zhang, S. Gimmig, G. Boucher, P. Wilkinson, Y. Shi, B. Yassine-Diab, E. A. Said, L. Trautmann, M. El Far, R. S. Balderas, M. R. Boulassel, J. P. Routy, E. K. Haddad, and R. P. Sekaly.** 2008. Transcription factor FOXO3a controls the persistence of memory CD4(+) T cells during HIV infection. Nat Med **14:**266-74.

281. **van Leeuwen, E. M., G. J. de Bree, E. B. Remmerswaal, S. L. Yong, K. Tesselaar, I. J. ten Berge, and R. A. van Lier.** 2005. IL-7 receptor alpha chain expression distinguishes functional subsets of virus-specific human CD8+ T cells. Blood **106:**2091-8.

282. **Varga, G., U. Dreikhausen, M. Kracht, A. Appel, K. Resch, and M. Szamel.** 1999. Molecular mechanisms of T lymphocyte activation:

convergence of T cell antigen receptor and IL-1 receptor-induced signaling at the level of IL-2 gene transcription. Int Immunol **11:**1851-62.

283. **Veazey, R. S., M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner.** 1998. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science **280:**427-31.

284. **Villinger, F., R. Miller, K. Mori, A. Mayne, P. Bostik, J. Sundstrom, C. Sugimoto, and A. Ansari.** 2004. IL-15 is superior to IL-2 in the generation of long-lived antigen specific memory CD4 and CD8 T cells in rhesus macaques. Vaccine **22:**3510-21.

285. **Viollet, L., V. Monceaux, F. Petit, R. Ho Tsong Fang, M. C. Cumont, B. Hurtrel, and J. Estaquier.** 2006. Death of CD4+ T cells from lymph nodes during primary SIVmac251 infection predicts the rate of AIDS progression. J Immunol **177:**6685-94.

286. **von Freeden-Jeffry, U., P. Vieira, L. Lucian, T. McNeil, S. Burdach, and R. Murray.** 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med **181:**1519-26.

287. **Vranjkovic, A., A. Crawley, K. Gee, A. Kumar, and J. Angel.** 2007. IL-7 decreases IL-7 receptor alpha (CD127) expression and induces the shedding of CD127 by human CD8+ T cells. Int Immunol **19:**1329-39.

288. **Vrisekoop, N., R. van Gent, A. B. de Boer, S. A. Otto, J. C. Borleffs, R. Steingrover, J. M. Prins, T. W. Kuijpers, T. F. Wolfs, S. P. Geelen, I. Vulto, P. Lansdorp, K. Tesselaar, J. A. Borghans, and F. Miedema.** 2008. Restoration of the CD4 T cell compartment after long-term highly active antiretroviral therapy without phenotypical signs of accelerated immunological aging. J Immunol **181:**1573-81.

289. **Walker, J. M., H. T. Maecker, V. C. Maino, and L. J. Picker.** 2004. Multicolor flow cytometric analysis in SIV-infected rhesus macaque. Methods Cell Biol **75:**535-57.

290. **Wan, Y. Y., H. Chi, M. Xie, M. D. Schneider, and R. A. Flavell.** 2006. The kinase TAK1 integrates antigen and cytokine receptor signaling for T cell development, survival and function. Nat Immunol **7:**851-8.

291. **Warby, T. J., S. M. Crowe, and A. Jaworowski.** 2003. Human immunodeficiency virus type 1 infection inhibits granulocyte-macrophage colony-stimulating factor-induced activation of STAT5A in human monocyte-derived macrophages. J Virol **77:**12630-8.

292. **Wherry, E., T. Becker, D. Boone, M. Kaja, A. Ma, and R. Ahmed.** 2002. Homeostatic proliferation but not the generation of virus specific memory CD8 T cells is impaired in the absence of IL-15 or IL-15Ralpha. Adv Exp Med Biol **512:**165-75.

293. **Williams, K., F. Hakim, and R. Gress.** 2007. T cell immune reconstitution following lymphodepletion. Semin Immunol **19:**318-30.

294. **Wojciechowski, S., P. Tripathi, T. Bourdeau, L. Acero, H. Grimes, J. Katz, F. Finkelman, and D. Hildeman.** 2007. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. J Exp Med **204:**1665-75.

295. **Wolfraim, L. A., T. M. Walz, Z. James, T. Fernandez, and J. J. Letterio.** 2004. p21Cip1 and p27Kip1 act in synergy to alter the sensitivity of naive T cells to TGF-beta-mediated G1 arrest through modulation of IL-2 responsiveness. J Immunol **173:**3093-102.

296. **Woodland, D. L., and J. E. Kohlmeier.** 2009. Migration, maintenance and recall of memory T cells in peripheral tissues. Nat Rev Immunol **9:**153-61.

297. **Yates, J., F. Rovis, P. Mitchell, B. Afzali, J. Tsang, M. Garin, R. Lechler, G. Lombardi, and O. Garden.** 2007. The maintenance of human CD4+ CD25+ regulatory T cell function: IL-2, IL-4, IL-7 and IL-15 preserve optimal suppressive potency in vitro. Int Immunol **19:**785-99.

298. **You, H., M. Pellegrini, K. Tsuchihara, K. Yamamoto, G. Hacker, M. Erlacher, A. Villunger, and T. W. Mak.** 2006. FOXO3a-dependent

regulation of Puma in response to cytokine/growth factor withdrawal. J Exp Med **203:**1657-63.

299. **Young, K. J., L. S. Kay, M. J. Phillips, and L. Zhang.** 2003. Antitumor activity mediated by double-negative T cells. Cancer Res **63:**8014-21.

300. **Zhang, M., J. Drenkow, C. S. Lankford, D. M. Frucht, R. L. Rabin, T. R. Gingeras, C. Venkateshan, F. Schwartzkopff, K. A. Clouse, and A. I. Dayton.** 2006. HIV regulation of the IL-7R: a viral mechanism for enhancing HIV-1 replication in human macrophages in vitro. J Leukoc Biol **79:**1328-38.

301. **Zhang, S. Y., Z. Zhang, J. L. Fu, F. B. Kang, X. S. Xu, W. M. Nie, C. B. Zhou, M. Zhao, and F. S. Wang.** 2009. Progressive CD127 down-regulation correlates with increased apoptosis of CD8 T cells during chronic HIV-1 infection. Eur J Immunol **39:**1425-34.

302. **Zhao, R., and R. Elder.** 2005. Viral infections and cell cycle G2/M regulation. Cell Res **15:**143-9.