The Role of Glucose Metabolism in T cell Stimulation and Homeostasis

by

Sarah Ruth Jacobs

Department of Pharmacology and Cancer Biology
Duke University

Date: _____

Approved:

_____
Jeffrey C. Rathmell, Supervisor

_____
Ann Marie Pendergast

_____
Tannishtha Reya

_____
Christopher Kontos

_____
Motonari Kondo

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Pharmacology and Cancer Biology in the Graduate School
of Duke University

2009

ABSTRACT

The Role of Glucose Metabolism in T cell Stimulation and Homeostasis

by

Sarah Ruth Jacobs

Department of Pharmacology and Cancer Biology
Duke University

Date: _____

Approved:

_____
Jeffrey C. Rathmell, Supervisor

_____
Ann Marie Pendergast

_____
Tannishtha Reya

_____
Christopher Kontos

_____
Motonari Kondo

An abstract of a dissertation submitted in partial
fulfillment of the requirements for the degree
of Doctor of Philosophy in the Department of
Pharmacology and Cancer Biology in the Graduate School
of Duke University

2009

Copyright by
Sarah Ruth Jacobs
2009

Abstract

The role of two cell extrinsic signals, T cell receptor (TCR) ligation and interleukin-7, in promoting glucose uptake and survival of T lymphocytes is examined in this work. Both of these signals are capable of regulating the uptake and fate of glucose, but the requirement of this regulation for T cell homeostasis and functionality remains unclear. To examine the role of TCR mediated increases of glucose metabolism and the signals involved, primary murine T cells were activated *in vitro* and the role and regulation of glucose uptake was examined. We show that glucose uptake is limiting in T cell activation and that CD28 costimulation is required for maximal glucose uptake following TCR stimulation by upregulating expression and promoting the cell surface trafficking of the glucose transporter Glut1. Regulation of T cell glucose uptake and Glut1 was critical, as low glucose prevented appropriate T cell responses. Additionally, transgenic expression of Glut1 augmented T cell activation, and led to accumulation of readily activated memory-phenotype T cells with signs of autoimmunity in aged mice. To further examine the regulation of glucose uptake, we analyzed CD28 activation of Akt, which appeared necessary for maximal glucose uptake of stimulated cells and which we have shown can promote Glut1 cell surface trafficking. Consistent with a role for Akt in Glut1 trafficking, transgenic expression of constitutively active Akt (mAkt) increased glucose uptake of resting T cells, but did not alter Glut1 protein levels. Therefore, CD28 appeared to promote Akt-independent upregulation of Glut1 protein and Akt-dependent Glut1 cell surface trafficking. In support of this model, co-expression of Glut1 and mAkt transgenes resulted in a synergistic increase in glucose uptake and accumulation of activated T cells *in vivo* that were largely independent of CD28.

Induction of Glut1 protein and Akt regulation of Glut1 trafficking are therefore separable functions of CD28 costimulation that cooperate to promote glucose metabolism necessary for T cell activation and proliferation.

Glucose uptake is dramatically increased in response to TCR and costimulation signaling, however, glucose uptake must be maintained at a low level in naive T cells to promote survival and homeostasis. Interleukin-7 (IL-7) plays a central role in maintaining naive T cell homeostasis, and mediates this effect *in vivo* at least in part through control of homeostatic proliferation and inhibition of apoptosis. IL-7 can promote glucose uptake and glycolysis *in vitro* and may also promote glucose metabolism *in vivo* to maintain T cell survival. To determine if IL-7 regulates T cell metabolism *in vivo*, we generated a transgenic model for conditional IL-7 receptor (IL-7R) expression on IL-7R$^{-/-}$ T cells. T cells in this model developed normally and, consistent with previous work, deletion of the IL-7R transgene *in vivo* led to cell death even in an otherwise normal lymphoid compartment. Importantly, *in vivo* deletion of IL-7R also led to decreased cell size and glycolytic flux. However, glucose uptake was not altered following deletion of the IL-7R indicating that while not essential for glucose uptake, IL-7 is required for maintenance of glycolysis. These data are the first to identify a signal required *in vivo* to regulate lymphocyte metabolism and demonstrate that in addition to its well-defined roles in homeostatic proliferation and cell survival, IL-7 plays a key and non-redundant role to maintain T cell glycolysis. Together, these data concerning the role of TCR, costimulation, and IL-7 in the regulation of glucose uptake and metabolism exemplify the importance of cell extrinsic signals and the regulation of glucose utilization.

Dedication

To Henry O'Neal Holifield and Diana Kate Sledge

Contents

Abstract iv

Contents vii

List of Figures xi

List of Abbreviations xiii

Acknowledgements xv

1. Introduction 1

1.1 Glucose metabolism 2

1.1.1 Glucose transporters 2

1.1.2 Points of regulation for Glut1 3

1.1.2.1 Regulation of Glut1 transcription and mRNA 3

1.1.2.2 Translational and post-translational control 4

1.1.2.3 Regulation of glucose metabolism beyond glucose uptake 9

1.1.3 Glucose metabolism and disease 10

1.2 T Lymphocytes 12

1.2.1 T cell development 13

1.2.2 Mature T cell function and life cycle 15

1.3 T cell stimulation 17

1.3.1 T cell stimulation signaling 17

1.3.2 Signaling of T cell costimulation 20

1.3.3 Stimulated T cell metabolism as a cancer model 22

1.3.4 T cell costimulation and glucose metabolism 25

1.4 T cell homeostasis 26

1.4.1 Factors that regulate homeostasis 27
1.4.1.1 TCR as a survival signal 27
1.4.1.2 IL-7 as a survival signal 28
1.4.2 IL-7 signaling 31
1.4.3 Regulation of T cell survival 33
1.4.3.1 IL-7 mediated regulation of glucose metabolism 33
1.4.3.2 IL-7 and Bcl-2 family members 34
1.5 Questions to be addressed 37
2. Materials and Methods 39
2.1 Mice 39
2.2 T cell purification and culture 39
2.3 Macrophage co-cultures 40
2.4 Proliferation, survival and flow cytometry 41
2.5 ELISA 41
2.7 Immunoblotting 42
2.8 Microscopy 43
2.9 Glucose uptake 43
2.10 Glycolysis 43
2.11 Hexokinase activity 44
2.12 Quantitative real time PCR analysis 44
2.13 Bone marrow reconstitution 45
3. Glucose Uptake is Limiting in T Cell Activation and Requires CD28-Mediated Akt Dependent and Independent Pathways 46
3.1 Introduction 46
3.2 Results 49

3.2.1 Costimulation via CD28 is necessary for increased glucose uptake upon T cell activation 49

3.2.2 Costimulation increases Glut1 protein levels and trafficking to the cell surface 51

3.2.3 Glut 1 protein levels are regulated by multiple pathways 54

3.2.4 Glucose is required for maximal immune response 56

3.2.5 Glut1 over-expression does not alter T cell development 59

3.2.6 Overexpression of Glut1 alters cell size and cytokine production 62

3.2.7 Chronic exposure to Glut1 over-expression results in increased T cell activation *in vivo* 62

3.2.8 Akt signaling plays a role in the regulation of glucose uptake but cannot fully substitute for CD28 signaling effects on glucose uptake 64

3.2.9 Glut1 and Akt additively increase T cell stimulation 68

3.3 Discussion 71

4. Interleukin-7 is essential to maintain T cell glycolysis *in vivo* 77

4.1 Introduction 77

4.2 Results 79

4.2.1 IL-7 regulates cell survival and cell death *in vivo* 79

4.2.2 Generation of an inducible IL-7R knockout system 81

4.2.3 IL-7R^{flox} transgene largely rescues IL-7R$^{-/-}$ phenotype 82

4.2.4 Loss of IL-7R decreases size, cell number, and signaling 85

4.2.5 Loss of IL-7R decreases cell survival 87

4.2.6 Growth and proliferation is delayed following loss of the IL-7R 90

4.2.7 Glycolysis is regulated by IL-7 signaling *in vivo* 92

4.3 Discussion 95

5. Conclusions and Future Directions 99

5.1 Induction of Glut1 protein following TCR ligation 100

5.2 Unchanged glucose uptake in IL-7R^{null} T cells 103
5.3 IL-7 and Bcl-2 family members 109
5.4 Cancer relevance 110
5.5 Final Remarks 112
References 114
Biography 136

List of Figures

Figure 1.1: Regulation of Glut1 5

Figure 1.2: T cell receptor signaling 19

Figure 1.3: IL-7R signaling 32

Figure 3.1: CD28 is required for maximal glucose uptake in T cell activation. 50

Figure 3.2: Costimulation induced Glut1 protein expression and trafficking to the cell surface. 53

Figure 3.3: Multiple pathways regulate glucose uptake following T cell stimulation. 55

Figure 3.4: T cells require glucose for cell survival, IL-2 production, proliferation, and IFN-γ production. 58

Figure 3.5: Glut1 over-expression in T cells does not alter T cell development or homeostasis in young mice. 60

Figure 3.6: Transgenic Glut1 over-expression increases T cell size and cytokine production. 61

Figure 3.7: Glut1 over-expression led to accumulation of memory-phenotype T cells in aged mice. 63

Figure 3.8: Constitutively active Akt increases glucose uptake in resting cells but cannot compensate for CD28 signals at low levels of CD3 stimulation. 67

Figure 3.9: T cell glucose uptake and activation are increased in Glut1 and mAkt double transgenic animals. 69

Figure 3.10: Model of TCR and costimulation effects on Glut1 72

Figure 4.1: IL-7 effects cell survival in adoptive transfer experiments. 80

Figure 4.2: Model of inducible IL-7R knockout. 82

Figure 4.3: IL-7R^{flox} transgene rescues T cell development in IL-7R$^{-/-}$ animals. 84

Figure 4.4: The IL-7R is efficiently excised. 86

Figure 4.5: Loss of IL-7R signaling effects cell survival. 89

Figure 4.6: Loss of IL-7R results in delayed stimulation. 91

Figure 4.7: IL-7R^{flox} transgenic T cells have metabolic responses similar to wildtype. 93
Figure 4.8: Loss of IL-7R reduced glycolysis rate *in vivo*. 94
Figure 5.1: How is Glut1 increased downstream of TCR signals? 102
Figure 5.2: Possible compensation signals following the loss of IL-7R 104
Figure 5.3: IL-7 signaling may regulate glycolytic enzymes. 107

List of Abbreviations

γ_c	common γ chain
APC	antigen-presenting cell
ARE	AU-rich element
CFSE	5,6-carboxyfluorescein succinimidyl diester
DN	CD4/CD8-double negative
DP	CD4/CD8-double positive
ELISA	Enzyme-linked immunosorbent assay
GIPC	G_α-interacting protein-interacting protein, C-terminus
GSK3	glycogen synthase kinase 3
HK	hexokinase
Ig	immunoglobulin
IL-2	interleukin-2
IL-2Rα	interleukin-2 receptor α-chain, CD25
IL-2Rβ	interleukin-2 receptor β-chain, also β-chain for IL-15R
IL-7	interleukin-7
IL-7R	interleukin-7 receptor
IL-7Rα	interleukin-7 receptor α-chain
ITAM	immunoreceptor tyrosine-based activation motif
Jak	Janus kinase
LAT	linker for the activation of T cells
LIP	lymphopenia-induced proliferation
MHC	major histocompatibility complex

mAkt	myristoylated Akt
mTOR	mammalian target of rapamycin
PIP	phosphatidylinositol phosphate
PI3K	phosphatidylinositol 3-kinase
PLCγ1	phospholipase Cγ1
RAPTOR	regulatory associated protein of mTOR
SP	single positive
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-related modifier
TCR	T cell receptor
T_h	helper T cell
TSC2	tuberous sclerosis 2
UTR	untranslated region

Acknowledgements

I would like to thank members of the Rathmell lab for their insightful comments, technical expertise, and teamwork. Some of the data included herein was produced in collaboration with Cathy Herman or Nancie MacIver. Equipment and technical expertise from the Duke Flow core and Duke transgenic mouse facility were greatly appreciated.

A special thanks to Jeff for being an excellent, patient, and understanding mentor. Thanks for believing in me and giving me an opportunity to study in your laboratory. I am also grateful to my other committee members Ann Marie Pendergast, Chris Kontos, Tannishtha Reya, and Motonari Kondo for their assistance with this work.

Thanks to Heather Wieman for all of her help and for sharing a bay with me for over three years; you are an excellent friend. I would also like to thank Brian Altman, Jon Coloff, Emily Ferguson, Pankuri Goraksha-Hicks, Cathy Herman, Nancie MacIver, Ryan Michalek, Jessie Wofford, and Yuxing Zhao for making the lab a productive yet fun place where I enjoy going. In addition to my fellow lab mates, without whom this work would not be possible, I would like to thank my parents and brother for their solid support and for making me who I am today. Finally, thanks to Nicholas— there is not space here to fully appreciate everything he has done.

1. Introduction

Cell extrinsic signals are required for cell survival during development and tissue homeostasis (Raff, 1992; Rathmell et al., 2000). In the absence of these signals or growth factors, cells undergo programmed cell death. One mechanism by which these social control signals may allow cells to evade apoptosis is maintenance of cellular metabolism. Acquisition of energy by individual cells in the form of sugars, lipids, or amino acids is critical for performing the housekeeping functions required for survival and biosynthesis for the production of necessary molecules (Krauss et al., 2001). Herein, we discuss the cell extrinsic signals that are known to promote the survival of T lymphocytes, T cell receptor ligation, and interleukin-7. Both of these signals have also been shown to regulate the uptake and fate of glucose (Frauwirth et al., 2002; Rathmell et al., 2001). Regulation of glucose utilization is of particular importance, as it is often deregulated and dramatically increased in cancer cells (Vander Heiden et al., 2009). Additionally, cancer cells are often independent of the signals normally required to avoid apoptosis, and therefore the study of cell extrinsic signal regulation of glucose metabolism is important for our understanding of how these pathways may be altered and targeted in cancer cells. We have used T lymphocytes as a model to study cell extrinsic signal regulation of glucose utilization because these cells are highly dependent on growth factor signals and dynamically regulate their glucose uptake and metabolism (Rathmell et al., 2003a; Rathmell et al., 2000; Vander Heiden et al., 2001).

1.1 Glucose metabolism

Glucose metabolism is often considered on the scale of the whole organism where the delicate balance of glucose homeostasis managed by the liver, muscle, and fat tissue is critical for the management of blood glucose levels and avoidance of diseases such as diabetes. However, glucose metabolism on a single cell level may be just as important for cell fate decisions. Cellular glucose metabolism may be regulated at many points, the first of which is the transport of glucose across the cell membrane. In mammalian cells, glucose is transported into the cell by a family of glucose transporter proteins called Gluts.

1.1.1 Glucose transporters

Glucose transporter proteins are divided into two families: facilitative, passive transporters and Na^+/glucose cotransporters. The facilitative, passive transporters transport glucose across the cell membrane and down a concentration gradient, and consist of thirteen members: Glut1-12 and HMIT (Joost et al., 2002). The second family, Na^+/glucose cotransporters, is dependent on sodium to actively transport glucose against the electrochemical gradient. It is composed of six or more members, but only SGLT1 and SGLT2 have been well characterized (Wright and Turk, 2004). All members of the facilitative glucose transporter family share a predicted structure of twelve highly conserved transmembrane segments (Mueckler et al., 1985; Mueckler et al., 1997). The proteins in the facilitative family of glucose transporters can be further divided into three classes. Glut1-4, which make up Class I, are the most characterized and have a large extracellular loop between the first and second transmembrane domain. Class II consists of GLUT5, 7, 9, and 11, which have the ability to transport fructose. Class III is composed of GLUT6, 8, 10, 12, and HMIT, which differ from class I in that their largest

extracellular loop is the ninth, rather than the first. However, in both cases the largest loop bears a glycosylation site (Zhao and Keating, 2007).

Each glucose transporter has unique expression, regulation, efficiency and kinetics. Na^+/glucose cotransporters are expressed mainly in the kidney and intestines. Facilitative glucose transporters, in contrast, are expressed throughout the body, but specific transporters vary in their expression by tissue. Our discussion will focus on the facilitative ubiquitous glucose transporter, Glut1. Glut1 is expressed in most tissues in the body, with the highest levels in the brain, where it is concentrated in the blood-brain barrier, and in the erythrocyte, eye, peripheral nerve, and lactating mammary gland (Zhao et al., 1993). Glut1 is also the primary glucose transporter in lymphocytes (Chakrabarti et al., 1994), the system that is the focus of the experiments detailed in Chapters 3 and 4. Therefore, the regulation of Glut1 will be discussed in detail below.

1.1.2 Points of regulation for Glut1

1.1.2.1 Regulation of Glut1 transcription and mRNA

Glucose transport or uptake is the first regulated step to acquire glucose from the environment, and the rate of transport into the cell can itself be modified by several factors. The earliest point of regulation affecting glucose uptake is through transcriptional control of the glucose transporter promoter. The Glut1 promoter contains a TATA box, two SP1 sites, and a phorbol ester responsive region (Angel et al., 1987; Dynan and Tjian, 1985). Early studies of the Glut1 promoter region with reporter constructs revealed that the promoter alone was insufficient to induce Glut1 transcription, and led to the identification of two enhancer elements within the Glut1 gene (Murakami et al., 1992). The Glut1 promoter can be inhibited by Sp3 interaction with the promoter and by unknown nuclear factor binding to a C8 box in the promoter

region (Fandos et al., 1999; Sanchez-Feutrie et al., 2003). Serum enhances the inhibitory effect of the C8 box, suggesting that growth factors are capable of regulating Glut1 transcription. Indeed, transcription may be regulated by serum-related growth factors PDGF, FGF, TGF-β, and hormones like growth hormone, thyroid hormone, insulin, and adipokines (MacIver et al., 2008; McGowan et al., 1995). Signaling pathways that integrate these extracellular stimuli to control the Glut1 promoter and transcription remain unknown.

In addition to transcriptional regulation, Glut1 protein levels may be modified through changes in RNA stability. Serum factors, small molecule inhibitors, and culture conditions have been shown to alter the half life of glut1 message (McGowan et al., 1995), and although the exact method of this regulation remains unclear, a potential mechanism has been proposed. The 3'UTR of glut1 contains an adenylate-uridylate rich element (ARE), which is an element known to confer variability in regulation of RNA stability through RNA binding proteins. Specifically, the RNA-binding protein HuR binds to glut1 mRNA and confers increased mRNA stability and accelerates the formation of translation complexes (Jain et al., 1997). Accordingly, siRNA of HuR resulted in decreased expression of Glut1 protein (Gantt et al., 2006).

1.1.2.2 Translational and post-translational control

In addition to regulation of transcription and RNA stability, Glut1 protein level may be regulated by mRNA translation and protein localization, degradation, and activity to alter the amount of glucose that is transported into the cell (Figure 1.1). Similar to the insulin responsive glucose transporter Glut4, regulation of Glut1 protein trafficking occurs through the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway (Welsh et al., 2005). Glut1 can respond to insulin, although not as robustly as Glut4, and it has been demonstrated that Akt increases expression and surface levels of

Glut1 in response to insulin (Piper et al., 1991). In mouse hepatoma cells, expression of a constitutively active Akt construct can increase glut1 transcription to levels similar to those seen in response to insulin treatment (Barthel et al., 1999), and in hematopoietic cells, constitutively active Akt partially maintained glut1 mRNA levels in the absence of growth factor (Plas et al., 2001). Treatment with LY294002, which blocks PI3K-dependent activation of Akt, reduced surface levels of Glut1 when total Glut1 protein levels were maintained on an exogenous promoter, indicating that the PI3K/Akt pathway may play a role not only in regulating the levels of Glut1, but also in subsequent regulation, such as the localization of the transporter (Wieman et al., 2007).

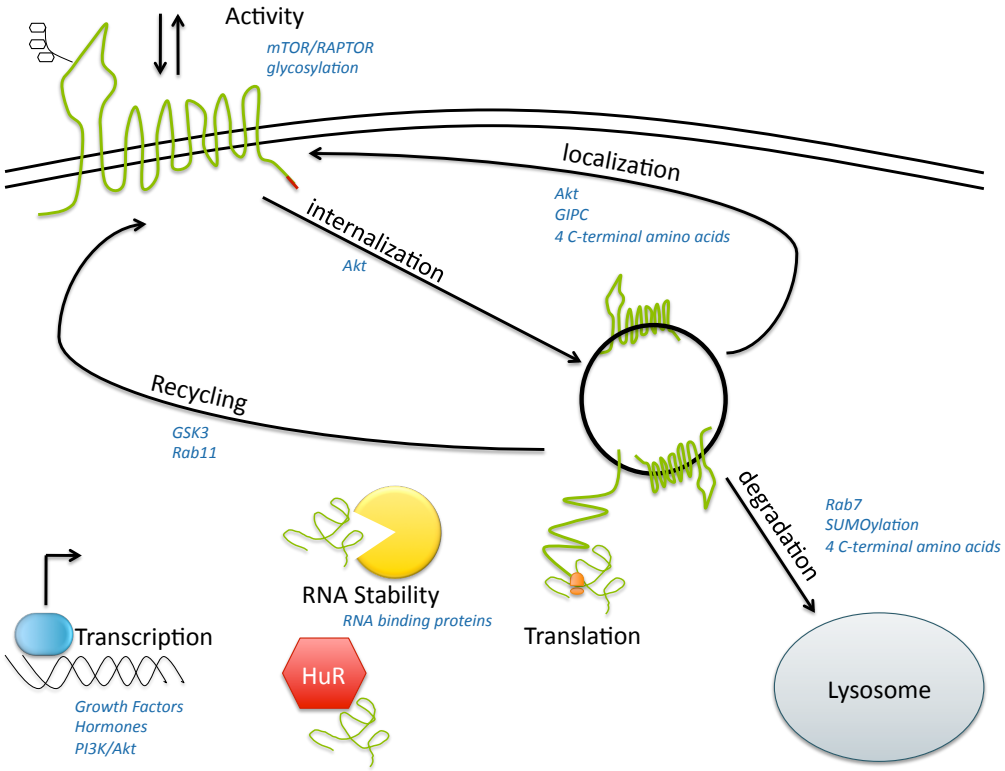

Figure 1.1: Regulation of Glut1

Glut1 can be regulated by the multiple different mechanisms outlined here. Factors shown in blue may affect the indicated steps including transcription, RNA stability regulation by HuR RNA binding protein, translation, localization to the cell membrane, activity that may be regulated by glycosylation shown on the largest extracellular loop, internalization that may be regulated by the C terminal 4 amino acids shown in red, recycling back to the membrane, and protein degradation.

Regulation of Glut1 surface levels by growth factors or the PI3K/Akt pathway was independent of protein synthesis, as addition of cyclohexamide to prevent protein synthesis neither prevented cytokine-mediated increases in Glut1 surface levels or glucose uptake, nor LY294002-mediated reduction in Glut1 surface levels. Fitting with the role of the PI3K/Akt pathway to regulate surface levels of Glut1, expression of a constitutively active Akt maintains Glut1 on the cell surface during growth factor withdrawal even when plasma membrane recycling was blocked, suggesting that Akt promotes maximal levels of surface Glut1 at least in part by preventing internalization. Ultimately this results in high levels of the transporter on the plasma membrane (Wieman et al., 2007).

Although localization of Glut1 to the cell surface is key for glucose transporter function, glucose uptake can be regulated even if the amount of transporter on the cell surface remains constant by controlling the rate of glucose transport. Evidence suggests that the glycosylation that occurs on the first extracellular loop of Glut1 is required for glucose to be transported across the membrane (Feugeas et al., 1991). Inhibition of this glycosylation by tunicamycin treatment or genetic mutation resulted in slower transport of glucose into the cell (Ahmed and Berridge, 1999; Asano et al., 1991; Asano et al., 1993; Onetti et al., 1997). These data suggest that glycosylation of Glut1 alters kinetic activity of the transporter.

In addition to altered glucose transport rates, multiple other signaling pathways have been implicated in the regulation of Glut1. Several signaling pathways activated downstream of Akt may account for the ability of PI3K/Akt to regulate Glut1 trafficking. Akt can activate the mammalian target of rapamycin (mTOR)/regulatory associated protein of mTOR (RAPTOR) (mTOR/RAPTOR) complex. This pathway has been shown to regulate the trafficking of multiple nutrient transporters, including the

transferrin receptor, low density lipoprotein receptor, and amino acid transporter 4F2 (Edinger and Thompson, 2002). As its name suggests, the mTOR/RAPTOR complex can be inhibited by addition of the small molecule rapamycin. Rapamycin treatment in 3T3-L1 adipocytes did not however alter insulin-stimulated Glut1 localization (Tremblay et al., 2005). In addition, rapamycin treatment of growth factor dependent hematopoietic cells did not alter surface or total levels of Glut1. Glucose uptake was however decreased, suggesting that the mTOR/RAPTOR complex did not affect trafficking but instead modified Glut1 transporter activity (Wieman et al., 2007).

Another signal downstream of Akt that may regulate glucose uptake is Akt phosphorylation of glycogen synthase kinase 3 (GSK3) to inactivate GSK3 kinase activity (Jope and Johnson, 2004). Pharmacological inhibition of GSK3 in growth factor-dependent hematopoietic cells did not alter Glut1 protein levels or internalization of Glut1, but nevertheless maintained Glut1 on the cell surface during cytokine withdrawal. This suggested that GSK3 regulated recycling of internalized Glut1 back to the cell surface (Wieman et al., 2007). In smooth muscle cells, inhibition of GSK3 resulted in an increase in Glut1 protein levels, which seemed to be dependent on signaling through the tuberous sclerosis 2 (TSC2)/mTOR complex, as a GSK3 inhibitor had no effect on $TSC2^{-/-}$ cells. This suggested that GSK3 inversely regulated glucose uptake by decreasing Glut1 protein levels in a TSC2/mTOR dependent manner in muscle cells (Buller et al., 2008). This contrasts with other reports that indicated GSK3 inhibition increased basal and insulin stimulated glucose uptake in skeletal muscle, but did not result in a change in Glut1 or Glut4 protein levels (Nikoulina et al., 2002). The different roles for GSK3 in these experiments may be due to examination of different time points, stimuli, or cell types. Regardless, it seems that GSK3 mediates glucose metabolism through regulation of glucose transporters in multiple tissues.

Proteins that regulate trafficking of intracellular vesicles and their formation may control glucose uptake by modulating the amount of Glut1 on the cell surface. Vesicle trafficking can influence the amount of transporter on the cell surface by modifying internalization, fusion with the plasma membrane, intracellular vesicle trafficking, and lysosomal fusion to destroy vesicle contents. Loss of Rab11, a Rab GTPase implicated in intracellular vesicle trafficking and recycling of intracellular vesicles to the cell surface, resulted in an accumulation of intracellular Glut1 (Ullrich et al., 1996), and decreased Glut1 levels on the cell surface (Wieman et al., 2007). A second small GTPase, Rab 7, has also been implicated in the regulation of glucose uptake by directing Glut1 containing vesicles to the lysosome for degradation. Loss of Rab7 protects nutrient transporters from degradation and promotes growth factor-independent cell survival (Edinger et al., 2003).

Genetic modification of the Akt pathway and Rabs reveals that control of Glut1 trafficking is regulated, but does not illustrate how the Glut1 protein interacts with these pathways. The C-terminal four amino acids of Glut1 appear to contain the signals or docking sites required for directed regulation of the transporter. These four amino acids are required for maximal expression and surface localization of Glut1, as their deletion or mutation resulted in Glut1 protein degradation (Wieman et al., 2009). The four terminal amino acids have also been implicated in binding the protein GIPC (G_{α}-interacting protein-interacting protein, C-terminus), which is also capable of binding to cytoskeletal elements and was therefore hypothesized to play a role in Glut1 trafficking (Bunn et al., 1999; Reed et al., 2005). GIPC depletion resulted in decreased glucose uptake, decreased surface levels of Glut1, and accumulation of Glut1 in intracellular vesicles that were distinct from those containing the transferrin receptor (Wieman et al., 2009). Additionally, GIPC was found to increase glucose uptake by promoting Glut1

trafficking to the cell surface (Wieman et al., 2009), suggesting that GIPC played a role in the regulation of Glut1 recycling. Specific regulation of Glut1 has emerged not only via the C terminal four amino acids, but also from the modification of lysine 456. Ubc9, a SUMO (small ubiquitin-related modifier) conjugating enzyme, has been implicated in the SUMOylation of this residue, to potentially target Glut1 for degradation (Giorgino et al., 2000; Wieman et al., 2009). These data clarify the regulation of intracellular localization, but there is a great deal more to understand. For example, recent evidence indicates that Glut1 is internalized via clathrin-independent endocytosis, a relatively uncharacterized form of endocytosis (Eyster et al., 2009). After internalization, vesicles fuse with late endosomes or lysosomes for degradation, or enter distinct tubular endosomes in order to recycle back to the plasma membrane. Identification of Glut1 as clathrin-independent endocytosis cargo will allow for further characterization of the regulation of both Glut1 and the control of clathrin-independent endocytosis.

1.1.2.3 Regulation of glucose metabolism beyond glucose uptake

Although glucose uptake is the first regulated step in cellular glucose metabolism, there are multiple points of regulation that determine the intracellular fate of glucose. The first of these is Hexokinase, which must phosphorylate glucose to prevent it from exiting the cell via the glucose transporters, which are bi-directional. Hexokinase can be regulated by its product glucose-6-phosphate, as well as by a variety of cell extrinsic signals, which may alter transcription or activity. Other steps of glycolysis are highly regulated as well, such as phosphofructokinase and pyruvate kinase. For example, phosphofructokinase-2 has been implicated as a target of Akt to maintain glycolysis and cell survival (Deprez et al., 1997). Glucose can be utilized in pathways other than glycolysis once inside the cell, such as hydrolysis through the pentose phosphate pathway to produce five carbon sugar intermediates and reducing

equivalents in the form of NADPH for biosynthesis reactions and redox control. The pentose phosphate pathway is primarily regulated by its first committed step, glucose-6 phosphate dehydrogenase. Glucose also has other potential roles in protein glycosylation or glycogen storage, although this pathway is not heavily used by lymphocytes.

1.1.3 Glucose metabolism and disease

Deregulation of glucose metabolism can result in deadly diseases. The quintessential diseases related to glucose metabolism, such as diabetes, result in defective glucose homeostasis. While Glut1 is not the main insulin-stimulated glucose transporter, polymorphisms in Glut1 have been implicated in susceptibility for type 2 diabetes (Makni et al., 2008). In addition to diabetes, there are other conditions associated with cellular glucose metabolism, including glucose deficiency syndrome and cancer.

Changes in glucose metabolism occur alongside oncogenesis, although it remains unclear if this is a cause or an effect of malignancy. The rapid growth and proliferation of cancer cells requires an increase in energy, which for many tumors results in increased usage of glucose. Cancer cells, however, do not fully oxidize glucose to produce the maximum amount of ATP, but undergo what has been termed aerobic glycolysis, where energy is derived from glycolysis and the production of lactate with limited oxidative metabolism despite the presence of abundant oxygen (Warburg, 1956). This metabolic phenotype is also known as the Warburg effect, and is used for cancer diagnosis in the clinic today with the application of fluorodeoxyglucose positron-emission tomography to identify tumors with high rates of uptake of this radiolabeled glucose analog (Gambhir, 2002).

In addition to HIF1α-mediated induction of glycolysis under hypoxic conditions (Lee et al., 2007), aerobic glycolysis is directly stimulated by oncogenes in cancer (Vander Heiden et al., 2009). Cancers with aberrant Akt activation demonstrate increased glycolysis and correlate with more aggressive malignancies (Elstrom et al., 2004). The oncogene Myc, a transcription factor, also plays a role in an increased ability of a cancer cell to acquire energy, as it activates transcription of many glycolytic enzymes, such as Hexokinase2 and Enolase (Kim and Dang, 2005). Additionally, a variety of oncogenes, including *src*, *ras*, *abl,* and the Fujinami Sarcoma Virus can regulate Glut1 transcription (McGowan et al., 1995). Recent evidence also implicates expression of an embryonic splice variant of Pyruvate Kinase in tumorigenesis, to promote a shift to aerobic glycolysis (Christofk et al., 2008). Although it is clear that many oncogenes and tumor suppressors lead to changes in glucose metabolism, it is not yet clear if increased energy availability leads to tumorigenesis, or is merely a byproduct of increased growth and replication. However, it is likely that interfering with this increased and deregulated metabolism will prove to be a fruitful anti-cancer therapy. Indeed, inhibition of glucose transporter expression by antisense RNA inhibited proliferation in a human leukemia cell line (Chan et al., 1999; Guenther et al., 2008).

Although metabolic targets are promising cancer treatments, creation of a drug that completely blocks glucose uptake will likely cause more harm than good. As previously mentioned, Glut1 supplies glucose to the brain and is ubiquitously expressed. The critical importance of Glut1 was demonstrated by an attempt to make Glut1 knockout animals, which resulted in blastocyst lethality (Heilig et al., 2003). Complete loss of Glut1 in humans has not been reported, however Glut1 deficiency syndrome or glucose transporter protein syndrome, in which patients suffer from a haplodeficiency of Glut1, has been characterized. This disease presents in childhood

with developmental impairment and seizures due to hypoglycorrhachia (Brockmann, 2009). The current treatment of Glut1 deficiency syndrome is a low carbohydrate, high fat diet to produce ketone bodies as an energy source for the brain, and results in a marked decrease in seizures and improvement in movement disorders. Glut1 has also been implicated in complications in development. As Glut1 is responsive to the glucose concentration of the cellular environment, hyperglycemia in pregnant mothers results in compensatory low glucose utilization prior to implantation of mouse embryos, and may be responsible for developmental retardation (Moley et al., 1998).

While Glut1 itself may not provide a suitable candidate for therapy, its regulation is clearly clinically important and understanding these processes may provide means to directly affect cancer cells in cancer therapy or activated lymphocytes in immunosuppression. The study of cellular glucose metabolism and its effects on development, diabetes, and cancer implicate glucose metabolism as a crucial factor for multiple conditions, and is worthy of further investigation.

1.2 T Lymphocytes

Tight control on glucose metabolism is critical, and regulation at a single cell level may be key for control of cell fate, immunosuppression, or treatment of cancer. Changes in glucose metabolism at the level of individual cells or groups of cells may alter cellular function and the ability to survive. Due to the metabolic changes that occur in T lymphocytes during activation and their requirement for cell extrinsic signals to survive, they are a malleable tractable model for examining metabolic control. T lymphocytes are an important part of the adaptive immune system and are capable of recognizing specific antigens. T lymphocytes derive their name from the place that they develop, the thymus, a small bi-lobed organ located near the heart. When T cells come in

contact with a foreign substance or antigen such as bacteria in the context of peptide/major histocompatibility molecules (MHC) on an antigen presenting cell, they become activated and induce changes in metabolism similar to what occurs during tumorigenesis.

1.2.1 T cell development

T lymphocytes are derived from hematopoietic stem cells, which can be found at the earliest stages of development in the liver and later on in the bone marrow. Cells destined to become T cells migrate to the thymus, where they undergo a rigorous selection process that requires the development of receptors on the surface of the T cell and cell extrinsic signals produced by non-lymphoid cells. T cells are defined by their expression of the T cell receptor (TCR), which is produced by a random process of DNA recombination between the V(D)J regions of the TCR locus. The random process allows for over 10^{18} unique TCRs and results in an ability to recognize tht many unique antigens (Market and Papavasiliou, 2003). However, V(D)J recombination produces some TCRs that are non-functional and TCRs that recognize self-antigens, both of which must be eliminated to make room for functional T cells and to prevent autoimmunity.

This selection process occurs in the thymus where the progression of T cell development can be followed by the expression of various cell surface markers. Upon entering the thymus, cells express neither CD4 nor CD8 and are therefore called double negative cells (DN). Double negative cells can be further dissected by their expression of CD25 and CD44 into four subsets. Cells are first CD44+ CD25- (DN1), then become CD44+ CD25+ (DN2), followed by CD44- CD25+ (DN3). DN3 is a particularly important stage during which cells of the α/β linage, a type of T cells categorized by the TCR they express, rearrange the TCR β chain by V(D)J recombination and must pass through the

first of multiple selections, β selection. T cells require a properly rearranged β chain and signaling through Notch, which interestingly has been shown to mediate cell size, glucose uptake, and metabolism, to successfully complete β selection (Ciofani and Zuniga-Pflucker, 2005). This suggests that developing thymocytes require cell extrinsic signals to maintain glucose metabolism during the selection process. Successful β chain rearrangement results in recombination at the α locus, and results in the production of an αβ TCR. Successful TCR rearrangement promotes down regulation of CD25 and pushes cells into CD25- CD44- (DN4), which is followed by expression of CD4 and CD8 that defines progression to the double positive (DP) stage of thymic development (von Boehmer and Fehling, 1997).

DP cells are tested for the production of a functional TCR by determining if they are capable of recognizing presented antigens. TCRs are not capable of recognizing free antigens, but instead detect antigen peptides presented on the major histocompatibility complex (MHC) of an antigen presenting cell. All mammalian nucleated cells express MHC class I on the cell surface and some cells also express MHC class II. MHC class I peptide complexes interact with CD8+ T cells and MHC class II with CD4+ T cells. Interaction between the TCR and MHC presenting a self-peptide results in functional TCR signaling to support positive selection and cell survival (Jameson et al., 1995). While association of a newly formed TCR to a self-antigen/MHC complex is required for positive selection and survival of DP thymocytes, if a TCR binds tightly to a self MHC, self-reactive T cells capable of inducing autoimmunity may be stimulated. Therefore, another selection is employed. In negative selection cells with a functional TCR that reacts strongly to self peptide presented on an MHC are eliminated by programmed cell death (Robey and Fowlkes, 1994). Less than 5% of DP thymocytes will endure the positive and negative selection processes and down regulate either CD4 or

CD8 to become a single positive (SP) thymocyte (Scollay, 1991). Single positive cells can be released into the periphery as mature T cells. While signaling though the TCR is a critical regulator of T cell development, other signals, including Notch and the cytokine interleukin 7 (IL-7), also play a role (Starr et al., 2003). The highly selective process of T cell development allows for maximal diversity of TCRs to increase the number of antigens that can be recognized by the T cell pool without allowing for the survival of autoimmune T cells.

1.2.2 Mature T cell function and life cycle

Once in the periphery, T cells circulate and interact with other immune cells within the secondary lymphoid organs. T cells are capable of long-term survival *in vivo*, where naive T cells may survive from months to years (Hellerstein et al., 1999; McCune et al., 2000; Michie et al., 1992). Removal from their normal microenvironment results in a rapid death and suggests that T cell are subject to survival control by social factors (Raff, 1992). Homeostatic cell extrinsic signals such as self-TCR activation and IL-7 are required for naive T cell survival (Jameson, 2002). When a T cell is presented with a foreign antigen MHC complex for which its TCR is specific and a costimulatory signal such as CD28, the T cell will undergo expansion and differentiate into effector cells. Expansion is accompanied by large increases in cell growth and rapid proliferation (doubling every 6-8 hours), both of which increase cellular energy demands in the process of inducing a T cell immune response. Effector T cell survival is regulated by TCR signaling and through the production of the cytokine IL-2 by activated T cells. However, activated T cells must be cleared from the body within days of activation to end inflammatory responses after the infection has dissipated. This occurs through both cell extrinsic and cell intrinsic apoptotic pathways (Weant et al., 2008). Cell extrinsic apoptosis is regulated by the cell surface receptor Fas and Fas ligand produced in an

autocrine fashion (Dhein et al., 1995). Fas receptor mutant mice fail to clear activated T cells, and ultimately develop autoimmune disease (Watanabe-Fukunaga et al., 1992). Although most activated cells must be removed, a small subset of antigen specific T cells survive and become memory T cells. While the precursor to the memory cell is still hotly debated, it is evident that both IL-7 and IL-15 are required for this process as well as for the long-term survival of memory T cells (Schluns and Lefrancois, 2003). Memory T cells allow for quick pathogenic clearance following repeated association with the same antigen.

The T cell life cycle described above is a simplification of a complex system. As previously mentioned, there are multiple kinds of T cells. Whereas the majority of T cells express an αβ TCR, some express a γδ TCR, and those cells have been shown to play an important role in the gut mucus and the skin. T cells expressing the αβ TCR chains can be further divided by expression of CD4 and CD8. CD4 positive T cells are helper T (T_h) cells that associate with MHC class II and serve mainly to produce cytokines and recruit other cell types to the site of infection. Helper T cells can differentiate into T_h1 or T_h2 cells characterized by production of IL-2 and IFNγ, or IL-4, respectively. In addition, regulatory T cells express CD4+, CD25+, and the transcription factor Foxp3 and, unlike most T cells, suppress immune responses to prevent damaging inflammation and autoimmunity. T_h17 cells, which also express CD4+, are distinguished by their production of IL-17 and play a pivotal role in inflammation. In addition to CD4+ T cells are the CD8+ T cells, or cytotoxic T cells, that associate with MHC class I. When activated, CD8+ T cells release factors that result in the apoptosis of target cells. These different T cell subsets work together to produce the cytokines and elicit inflammation or apoptosis required for an effective adaptive immune response.

1.3 T cell stimulation

For T cells to perform their function during pathogenic responses, the TCR must recognize the antigen presented via MHC and translate this interaction intracellularly to initiate proliferation, differentiation, cytokine production, and in some cases memory cell formation. This is accomplished by generation of a large signaling complex, the involvement of many major signaling pathways, and requires a dramatic increase in energy expenditure.

1.3.1 T cell stimulation signaling

While TCR/MHC interaction is obligatory for T cell activation, there are a multitude of other signals involved in the interaction between an APC and a T cell to elicit successful T cell activation. A costimulatory signal, the best characterized of which is CD28 binding to CD80 or CD86 (B7.1 or B7.2) on the APC, is required for most responses to peptide antigens (Bretscher and Cohn, 1970; Rudd et al., 2009). TCR stimulation in the absence of CD28 ligation results in anergy, an arrested state of unresponsiveness, or in some cases cell death (Jenkins and Schwartz, 1987; Lucas et al., 1995; Webb et al., 1990). Opposite to CD28, there are also receptors on the T cell that interact with proteins on the APC that curtail T cell activation such as CTLA-4 and PD-1 (Riley and June, 2005). In addition, other proteins involved in the site of interaction between an APC and T cell include the coreceptors CD4 or CD8 and LFA-1, an adhesion molecule that interacts with ICAM-1 (Dustin, 2009). The clustering of the TCR, coreceptors, and adhesion molecules within a T cell is critical for the formation of an intracellular signaling complex. This complex amplifies the antigen signal and induces the gene transcription necessary to produce an immune response.

Although TCR signaling has been determined to be a non-linear process, a simplification of TCR signaling will be attempted here (Figure 1.2). Firstly, the TCR complex is composed of several different proteins. These include the classical αβ chains that confer antigen specificity to the T cell, as well as the γ, ε, δ, and ζ chains, which together comprise CD3. The CD3 component of this receptor complex is responsible for initiating intracellular signaling and antibody induced clustering of the CD3 portion of the TCR complex can be used to mimic TCR antigen recognition. The intracellular tails of the CD3 proteins and ζ chains contain motifs designated immunoreceptor tyrosine-based activation motifs (ITAMs) that are phosphorylated upon TCR ligation by the Src family kinases Lck and Fyn. Phosphorylation of ITAMs results in recruitment of ZAP-70, a tyrosine kinase of the Syk family. This initiates phosphorylation of LAT (linker for the activation of T cells), a transmembrane adopter protein, and SLP-76 (Src homology 2 (SH-2) domain-containing leukocyte phospho-protein of 76kDa), a cytosolic adaptor protein. These two adaptor proteins form the foundation of TCR signaling, as loss of either protein results in a near complete loss of T cell activation (Koretzky et al., 2006; Zhang et al., 1999). After phosphorylation, LAT can in turn activate PLCγ1 (phospholipase Cγ1), PI3K, GRB2 (growth factor receptor-bound protein 2), and Gads (GRB2-related adaptor downstream of Shc). SLP-76 interacts with Vav1, Nck, Itk (IL-2-induced tyrosine kinase), Gads, PLCγ1, ADAP (adhesion and degranulation-promoting adopter protein), and HPK1 (hematopoietic progenitor kinase 1). These interactions are the final steps in what has become known as proximal TCR signaling and lead into the initiation of multiple critical intracellular signaling cascades. Activation of PLCγ1 results in formation of IP_3 and DAG (diacylglycerol). In turn DAG activates RAS, and therefore MAPK (mitogen-activated protein kinase) signaling, as well as PKCθ (protein kinase Cθ) and its downstream activation of NF-κB. IP_3 initiates Ca^{2+} release from the endoplasmic

reticulum and activates the NFAT (nuclear factor of activated T cells) transcription factor. Other major signaling pathways activated by TCR ligation include weak activation of the PI3K/Akt pathway downstream of Lck and Ras, modulation of actin cytoskeletal elements though ZAP-70 recruitment of the WASP-WIP complex, and Vav1 mediated activation of Rac. These signaling cascades all play critical roles in induction of intracellular TCR signals and are regulated by complex protein-protein interactions of the enzymes and adaptors present in TCR proximal signaling (Smith-Garvin et al., 2009).

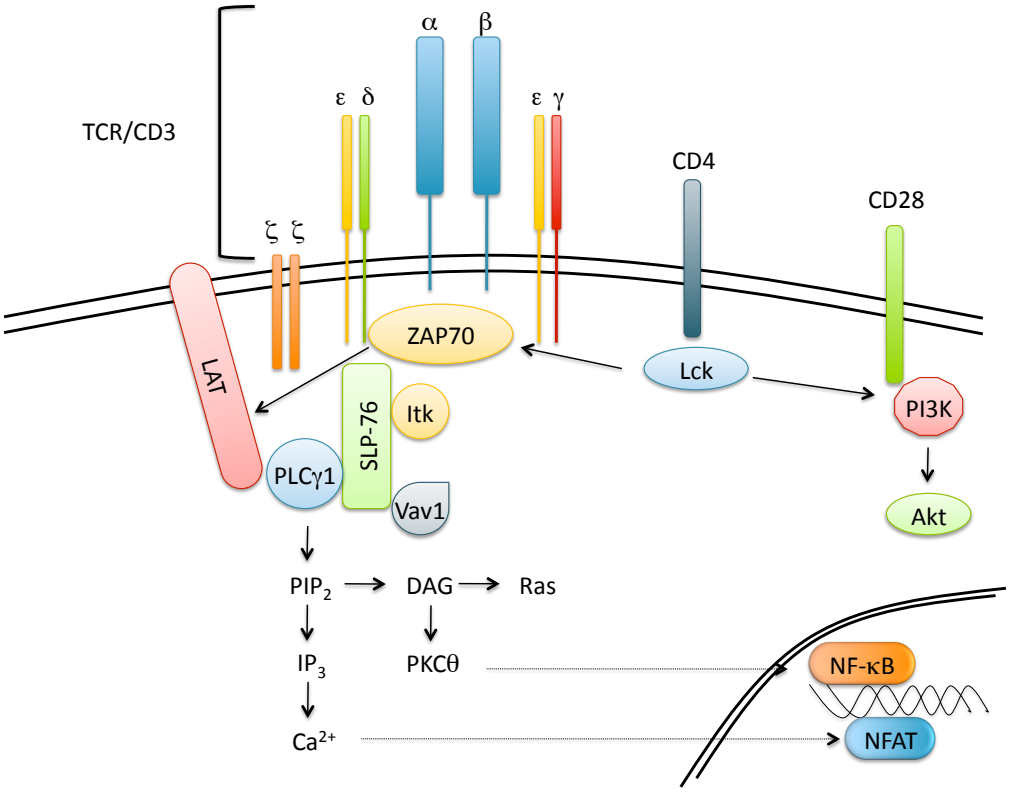

Figure 1.2: T cell receptor signaling

T cell receptor signaling occurs through the TCR/CD3 and cooperation between the CD28 costimulation receptor and coreceptors CD4 or CD8. Interaction of these receptors with their ligand on an antigen presenting cell activates the signaling cascade indicated here and results in the activation of multiple major signaling pathways including Ras, PKCθ, Akt, and the transcription factors NF-κB and NFAT.

1.3.2 Signaling of T cell costimulation

As previously mentioned, even though TCR ligation activates and integrates all of the above signals, a second or costimulatory signal must be received for T cells to mount an immune response. The most characterized of these costimulatory signals is CD28 ligation to CD80 or CD86. There is controversy surrounding the role of CD28 and whether it produces a distinct signal or merely augments the signals produced by the TCR. While historically thought to initiate a distinct signaling pathway, genomic and transcriptional expression profiling does not reveal unique signals and instead suggests that CD28 serves mainly to increase or alter the signaling pathways activated by the TCR (Diehn et al., 2002; Riley et al., 2002). Although no unique signaling initiation by CD28 has been pinpointed, it remains a possibility that regulation of specific pathways by CD28 may qualitatively alter downstream TCR signaling.

Specific pathways known to be augmented by CD28 coligation include PI3K/Akt (August et al., 1994), increased cytosolic free calcium (June et al., 1990), activation of Jnk (Su et al., 1994), Ras, and MAPK (Nunes et al., 1994). The mediators of these pathways that may be responsible for altered signals downstream of CD28 include PI3K, Tec kinases such as Tec and Itk, and Vav1. When the cytoplasmic tail of CD28 is phosphorylated, it can bind the p85 subunit of PI3K (Pages et al., 1994). Activation of PI3K results in production of PIP_2 and PIP_3 and activation of the phosphoinositide-dependent protein kinase 1 (PDK1), which subsequently promotes activation of the protein kinase Akt. The increased signaling through the Akt pathway afforded by CD28 ligation prevents cell death through increased expression of the anti-apoptotic protein Bcl-xL (Boise et al., 1995; Burr et al., 2001), increased protein synthesis via signaling through mTOR (Cantrell, 2002), and increased cellular metabolism (Frauwirth et al., 2002)— a topic that will be discussed in detail in section 1.3.4. Activation of PI3K may

also indirectly increase NFAT signaling through inhibition of glycogen synthase kinase 3 (GSK3), a well-described target of Akt. GSK3 promotes export of NFAT from the nucleus and activated Akt inhibits GSK3, therefore signaling through PI3K/Akt increases the ability of NFAT to remain in the nucleus and transcribe target genes (Beals et al., 1997).

CD28-stimulated formation of PIP_3 at the plasma membrane also recruits the Tec kinases Itk, Tec, and Rlk to the immunological synapse (August et al., 1994; Berg et al., 2005). Tec and Itk are critical regulators of PLCγ1 and therefore contribute to calcium signaling, NFAT, and signaling pathways downstream of DAG such as the Ras/Raf/Erk pathway. In addition to playing a role in these signaling pathways, Tec and Itk also regulate WASP to control nucleation of actin filaments and remodeling of the cytoskeleton. T cells deficient in Itk have reduced TCR responses, a phenotype distinct from CD28 knockout animals, which have normal proximal TCR signaling, suggesting that Itk and CD28 have unique roles (Liao and Littman, 1995). Evidence for a regulatory role of Itk was demonstrated by increased proliferation of Itk-deficient T cells after TCR stimulation. This suggested that Itk may regulate TCR and CD28 signals to decrease signal strength (Liao et al., 1997). T cells deficient in Itk are still capable of activating most CD28 signals, therefore, while performing an important role, Itk may not be critical for costimulation (Li and Berg, 2005).

Another factor that may mediate CD28 specific signals is Vav1. Vav1 is part of a cytoplasmic family of guanine nucleotide exchange factors (GEFs) for Rho-family GTPases and has been shown to regulate the activity of Rac1 and possibly Cdc42 (Rudd et al., 2009). Vav1 is thought to be an important mediator of CD28 signals, as it can be co-immunoprecipitated with CD28 (Schneider and Rudd, 2008), and aids in membrane anchoring independent of PI3K (Michel and Acuto, 2002). Phosphorylation of Vav1 results in an increase in GEF activity to regulate the actin cytoskeleton (Crespo et al.,

1997; Han et al., 1997) that serves not only to propagate structural changes, but to facilitate clustering of the CD3 complex to increase TCR proximal signals (Krawczyk et al., 2002). Additionally, Vav1 increases CD28 induced activation of NFAT and NF-κB (Acuto and Michel, 2003), as Vav1 deficiency results in a partial reduction in intracellular calcium flux, activation of the ERK/MAPK pathway, and activation of NF-κB (Costello et al., 1999; Fischer et al., 1998; Holsinger et al., 1998). There is evidence that Vav1 influences nearly all of the TCR signaling pathways (Turner and Billadeau, 2002), and that its major mechanism of enhancing CD28 is to work with SLP-76 to create a strong signaling complex for the TCR (Michel et al., 2000; Tuosto et al., 1996). SLP-76 is required for Vav1 activation downstream of the TCR, but not for CD28, suggesting that these receptors may recruit Vav1 differently (Acuto et al., 2003; Myung et al., 2001). Indeed, CD28 mutants that cannot activate Vav1 affect SLP-76, PLCγ1, and Itk phosphorylation, and exhibit a phenotype very similar to Vav1 deficiency, thus supporting a role for Vav1 as a crucial integrator of TCR and CD28 coligation (Michel et al., 2001; Reynolds et al., 2002).

1.3.3 Stimulated T cell metabolism as a cancer model

Interaction of a TCR with an antigen presented via MHC, accompanied by costimulatory signals, activates the major signaling pathways outlined above to elicit an immune response. The effects of activation on a T cell depend on the cell type. Helper T cells must dramatically increase cytokine production and cytotoxic T cells synthesize the enzymes needed to kill infected cells. To accomplish these tasks, stimulated T cells must first increase in size and proliferate to increase the number of cells responding to the specific antigen. These activities require energy not only for growth, but also for the synthesis of macromolecules, including proteins, nucleic acids, and excreted factors.

While a great deal of effort has been expended understanding the signaling pathways involved in TCR stimulation, how metabolism is regulated in these cells is poorly understood.

Resting mature T cells in the periphery exist in a quiescent state ideal for the extended life of these cells. In this condition, T cells use low levels of glucose and other essential nutrients (Hume et al., 1978; Tollefsbol and Cohen, 1985) to perform housekeeping functions such as the maintenance of ionic gradients, volume regulation, and cell growth (Buttgereit et al., 2000). Even this basal level of nutrient uptake requires cell extrinsic signals, as loss of these signals results in decreased glucose uptake and eventual T cell death (Rathmell et al., 2001; Rathmell et al., 2000). Basal glucose utilization is split between the metabolic pathways of glycosylation reactions, lactate production through glycolysis, and oxidation (Hume et al., 1978). Resting T cells produce nearly 85% of their ATP from oxidation. Following T cell stimulation, however, proliferating cells derive the majority of their ATP from glycolysis (Brand and Hermfisse, 1997).

Energy in the form of ATP is necessary for multiple specific immune functions including migration, phagocytosis, cytokine production, and effector function (Buttgereit et al., 2000). Mitogenic stimulation of T cells is accompanied by a rapid increase in glucose usage and lactate production with only a moderate increase in oxygen consumption (Bental and Deutsch, 1993; Hume et al., 1978; Roos and Loos, 1973). Lactate production via an anaerobic pathway even in the presence of oxygen has been termed aerobic glycolysis and was originally described in cancerous cells (Warburg, 1956). This shared metabolic program between activated T cells and tumor cells defines T cells as an excellent model in which to study malignant cell metabolic regulation. Examination of metabolism in activated T cells is more advantageous than in tumor cells

due to the karyotypic normality and lack of mutation in T cells compared to the average cancer cell. Therefore, T cells allow for the study of metabolic regulation in non-transformed cells and how these pathways may be altered in transformed cells to promote cancer cell metabolism.

It remains unclear why aerobic glycolysis is preferred as a metabolic program in T cell activation or in cancer cells. Glycolysis at such high levels may be mutually exclusive with oxidative metabolism; enhanced recycling of NAD+ to NADH that accompanies glycolytic induction can compete with NADH usage in the TCA cycle (Greiner et al., 1994). Alternatively, aerobic glycolysis may be essential for the rapid growth necessary for an immune response by providing biosynthetic precursors for lipid, amino acid, and nucleic acid synthesis (Fox et al., 2005). Additionally, aerobic glycolysis may be associated with kinetics and the speed at which ATP is required. ATP production may be more rapidly produced via glycolysis than through oxidation. To support this, evidence reveals that as quickly as 15 minutes following T cell activation, AMPK (AMP-activated protein kinase) is activated, and although energy expenditure is not increased at this time point following TCR ligation, AMPK anticipates the increased need for energy and reduces ATP consumption and increases ATP production (Tamas et al., 2006). This early activation of energy producing and conserving pathways, even in the presence of ample energy, suggests that T cells may require ATP very early on for maximal function.

Although glucose is important as an energy source, other energy and carbon sources are available to potentially substitute for glucose. The amino acid glutamine has been designated as a critical nutrient for hematopoietic cells, although replacement of glucose with glutamine is not sufficient to substitute for the carbon or energy needed

during stimulation or glucose needs of the endoplasmic reticulum, and T cells fail to proliferate (Greiner et al., 1994).

1.3.4 T cell costimulation and glucose metabolism

How glucose metabolism is increased during a T cell response is still under investigation. Several signaling pathways have been implicated in the regulation of metabolism including Ca^{2+} levels, calcineurin, intracellular pH, ATP levels, PKC, and MAPK (Krauss et al., 2001). Transcriptional activation though NFAT has also been suggested, as this pathway is initiated by TCR ligation and has been shown to play a role in insulin stimulated glucose uptake in adipose tissues (Yang et al., 2006). While the role of these pathways in the regulation of metabolism is still being confirmed, signaling through the costimulatory receptor has emerged as an important regulator of increased glucose metabolism during T cell activation (Frauwirth et al., 2002). In human primary T cells, it was demonstrated that CD28 coligation is required for increased glucose uptake and glycolysis. This was not due to CD28 mediated increases in IL-2 production, and induction occurred at a level sufficient to suppress oxidative metabolism and promote aerobic glycolysis. CD28 costimulation increased Glut1 expression in a PI3K dependent fashion, and expression of a constitutively active Akt in a lymphoid cell line resulted in increased glycolysis and glucose uptake.

These data suggested that costimulation signaled through the PI3K/Akt pathway to increase glucose uptake during T cell activation. This trend has been observed in other cell types, particularly other immune cells, which must convert from a quiescent phenotype into fast acting defenders. B cells, the other main cell type in the adaptive immune system, rapidly increase glucose uptake and glycolysis when their antigen specific receptor is engaged (Doughty et al., 2006). Similar to T cells, the increase in glucose utilization was found to rely upon the PI3K/Akt signaling pathway, as

treatment with PI3K inhibitors decreased glucose uptake and glycolysis, and activation of Akt alone increased glucose utilization. Increased glucose use has also been demonstrated in cells of the innate immune system in response to activating cytokines. For example, colony stimulating factor 1 (CSF-1) treatment of CSF-1 dependent macrophages resulted in increased glucose uptake and Glut1 surface expression, and was dependent on PI3K and PLC signaling, as well as their downstream effectors Akt and PKC (Chang et al., 2009). Finally, although not responding to an antigenic stimulus, cytokine-dependent cells, when deprived of this cytokine, cease to uptake glucose and fail to survive, indicating that cytokine receptors and TCR may regulate similar pathways to mediate glucose metabolism (Kan et al., 1994; Rathmell et al., 2001). This cell extrinsic regulation of glucose uptake and metabolism will be explored in the next section.

1.4 T cell homeostasis

Glucose metabolism may be critical not only for activated T cells, but also in the maintenance of naive T cells. Regulation of T cell numbers in the lymphocytic pool is vital for proper function of the adaptive immune system and may be regulated by cell extrinsic factors. Low numbers of T cells reduce the diversity of available T cell receptors and a corresponding decrease in the number of foreign antigens capable of being recognized and defeated. However, T cell numbers must be limited within the physical space of the secondary lymphoid organs. For these reasons, the immune system has evolved to tightly regulate the number of T lymphocytes within the immune compartment. Different subsets of T cells, such as naive cells, activated cells, CD4 memory cells, CD8 memory cells, and regulatory T cells, appear to be regulated by

individualized survival signals. CD4 and CD8 naive T cells share the same cell survival cues and which consist mainly of low levels of TCR activation and the cytokine IL-7.

1.4.1 Factors that regulate homeostasis

Naive T cells fail to survive in standard culture conditions for more than two days without additional signals. This suggests that cell extrinsic signals available *in vivo* must be actively promoting cell survival. Several factors have been implicated in the regulation of naive T cell survival, most notably self-antigen TCR stimulation and the cytokine IL-7.

1.4.1.1 TCR as a survival signal

Signaling through the TCR is required for thymic selection and plays a role in mature T cell survival. When deprived of self-peptide MHC, the life span of both CD4 and CD8 T cells is decreased (Takeda et al., 1996; Tanchot et al., 1997). The role of TCR signaling in naive T cell homeostasis has been confirmed with conditional loss of signals proximal to the receptor such as Fyn and Lck, which resulted in reduced survival of both CD4 and CD8 T cells (Seddon and Zamoyska, 2002). When T cells are stripped of their TCRs by conditional genetic ablation, half life of naive T cells decreased by more than half that of T cells that retain their TCR (Labrecque et al., 2001; Polic et al., 2001). These results have outlined an important role for TCR self antigen signals for the maintenance of naive T cell survival and established the strength of signal necessary to maintain survival without inducing proliferation or autoimmunity. TCR signals are not, however, the only cell extrinsic signal required to keep naive T cells alive, and other signals may account for the gradual death of T cells without TCRs compared to B cells with deleted B cell receptors.

1.4.1.2 IL-7 as a survival signal

A second major signal required for naive T cell homeostasis is the cytokine IL-7. Although identified as a pro-B cell survival factor (Namen et al., 1988), IL-7 was first studied for its role in lymphopoiesis. IL-7 is required for T and B cell development in mice and T cell development in humans. The cytokine also has a role in T cell homeostasis and lymphopenia induced proliferation. IL-7 is produced by non-lymphoid stromal cells in several tissues including thymus, lymphoid organs, liver, skin, intestines, and bone marrow, with the thymus having the highest levels of IL-7 production of any organ (Wiles et al., 1992). More recent work has indicated that IL-7 production in lymphoid organs is accomplished mainly by a subset of fibroblastic reticular cells that are located within T cell zones, and these cells alone are capable of maintaining T cell survival *in vitro* (Link et al., 2007). IL-7 is detected by T cells through the dimerization of the common gamma chain (γ_c) that is shared with IL-2, IL-4 IL-9, IL-15, and IL-21, and the IL-7Rα (Puel et al., 1998), which recognizes IL-7 and the related cytokine, thymic stromal lymphopoietin.

Production of IL-7 appears to occur largely at a constant rate and modulation of the IL-7 signal is achieved through regulation of the IL-7R. The IL-7Rα is expressed early in lymphoid cell development by common lymphoid progenitor cells in the bone marrow which can develop into both T and B cells (Kondo et al., 1997b). While IL-7R expression remains high during most stages of T cell development, it is down regulated at important points of growth and selection. For example, sustained IL-7R expression in common lymphoid progenitors impairs B cell development to sustain pro-B cell proliferation (Purohit et al., 2003) and IL-7R down regulation occurs when thymocytes progress from the double negative to double positive stage of T cell development. Only cells that undergo positive selection re-express the IL-7R and become mature T cells

(Mazzucchelli and Durum, 2007). The down regulation of the IL-7R at this stage of development may force thymocytes to derive a survival signal from a mediator other than IL-7, such as the TCR, to ensure proper T cell selection. Alternatively, down-regulation of the IL-7R may serve to increase the amount of IL-7 available to other subpopulations within the thymus.

It has been hypothesized that as IL-7 is produced by non-lymphoid cells and remains constant, the amount of available cytokine decreases as it is bound to cells to provide a survival signal, thus producing competition for IL-7 amongst lymphocytes. The greater the number of T cells that are present, the fewer are able to obtain the signal, and conversely, in a lymphopenic environment, the amount of available IL-7 increases as T cell numbers decline (Bolotin et al., 1999). This rheostat-like function of IL-7 is supported by the down-regulation of the IL-7R through T cell activation and IL-2 induced suppression (Schluns et al., 2000; Xue et al., 2002). This forces activated T cells to derive a survival signal from elsewhere, leaving IL-7 available to provide for the survival of naive T cells. The rheostat model of IL-7 signaling also applies to naive T cells, as interaction of T cells with IL-7 results in a decrease in the amount of IL-7R on the cell surface, possibly to ensure that cells that have received the survival signal do not continue to bind IL-7, and allow for the maximum number of cells to survive (Park et al., 2004; Vranjkovic et al., 2007).

Further support of the rheostat role of IL-7 maintenance of the naive T cell pool comes from multiple studies in which the levels of IL-7 have been increased *in vivo*. Administration of endogenous IL-7 in mice was found to effectively increase lymphocyte numbers as well as T cells, B cells, and specific populations of progenitor cells by what was identified as a non-activating process (Faltynek et al., 1992; Geiselhart et al., 2001; Komschlies et al., 1994). An increase in T cells was also observed in humans

that were treated with exogenous IL-7 (Rosenberg et al., 2006). Furthermore, increasing levels of IL-7 through genetic modulation resulted in increased numbers of thymocytes, mature T cells (Samaridis et al., 1991), and the development of lymphoproliferative disorders that ultimately induced the formation of lymphomas (Rich et al., 1993).

Study of the role of IL-7 in naive T cells through the loss of the signal has been technically difficult, as both IL-7$^{-/-}$ and IL-7R$^{-/-}$ animals have demonstrated that IL-7 is imperative during early stages of T cell development. These animals exhibit a severe reduction in the number of thymocytes beyond the double negative stage and of mature T cells (Peschon et al., 1994; von Freeden-Jeffry et al., 1995). Similarly, humans with defective IL-7R expression or signaling demonstrate T-B+NK+ severe combined immunodeficiency (Puel et al., 1998). Much has been clarified on the role of IL-7 in T cell development and further discussion of this role of IL-7 is beyond the scope of this work. To study IL-7 effects on mature naive T cells, IL-7 signaling has been ablated by injection of blocking antibodies into animals. This approach resulted in a complete inhibition of the development of B cell progenitors and a significant decrease in thymic cellularity. In this study no effect was observed on the number of mature T cells (Grabstein et al., 1993). This may have been due to an inability of the antibody to fully block IL-7 signals. More recent attempts to neutralize IL-7 signals by antibody injection have, however, resulted in a 50% reduction in T cell numbers after seven days (Wojciechowski et al., 2007). Adoptive transfer experiments have also supported the role of IL-7 as a regulator of naive T cell homeostasis. Transfer of wildtype T cells into IL-7$^{-/-}$ animals resulted in shortened T cell lifespan (Schluns et al., 2000; Tan et al., 2001). Survival of the limited number of mature T cells in IL-7R$^{-/-}$ mice is also decreased, but this result is complicated by the potential for altered development and selection of T cells that developed without IL-7 (Maraskovsky et al., 1996; Schluns et al., 2000). All methods of determining the role

of IL-7 in naive T cell homeostasis utilized thus far have been performed in altered environments, such as lymphopenic or by inhibiting access to IL-7 in all cells, and have yet to define a clear cell intrinsic role for IL-7 in maintaining T cell survival. Importantly, in addition to the regulation of naive T cell homeostasis, adoptive transfer experiments have also demonstrated that IL-7 plays a critical role in the regulation of homeostatic proliferation, or lymphopenia-induced proliferation (LIP)— a dramatic increase in proliferation in lymphocytes upon introduction into a T cell depleted environment.

In addition to its requirements during LIP, naive T cell survival, and T cell development, availability of IL-7 has also been indentified as a key factor for the survival of memory cells (Surh et al., 2006). Therefore, IL-7 is a unique cytokine that plays important roles in multiple stages of lymphocyte development and regulation and is critical for the maintenance of multiple T cell subpopulations. It remains unclear how IL-7 mediates these functions.

1.4.2 IL-7 signaling

As IL-7 is a key factor for lymphocytes, there has been much interest in the mechanisms through which IL-7 mediates its signals to allow for development, survival, and proliferation. The IL-7R targets multiple downstream signaling pathways through dimerization with the γ_c. Mutation of the γ_c results in an inability to transduce cytokine signals including IL-7 and is often associated with X-linked severe combined immunodeficiency in humans (Uribe and Weinberg, 1998). Dimerization between the γ_c and IL-7R recruits the protein tyrosine kinase Jak3, a member of the Janus kinase family, which phosphorylates the γ_c and Jak1, resulting in the creation of docking sites for STAT transcription factors 1, 2, 3, and 5 (Figure 1.3). Subsequent phosphorylation of the STATs by Jak1 or Jak3 results in their homo- or hetero- dimerization and translocation to the

nucleus, where they activate transcription of target genes. The roles of these signaling factors have been clarified through the production of knockout animals such as Jak3 deficient mice, which demonstrate a severe block in thymocyte development, but after time produce immune systems with a normal number of T cells (Baird et al., 1998). Jak1 deficient mice also have impaired thymic development and responses to IL-7, indicating that both Jak1 and Jak3 are essential (Rodig et al., 1998).

Although STAT1 , 2, and 3 can be activated by IL-7, loss of these transcription factors does not result in defects in T cell development, indicating that they are not required for IL-7 signaling (Durbin et al., 1996; Park et al., 2000; Takeda et al., 1998). Deletion of both isoforms of STAT5 (STAT5a and STAT5b), however, resulted in a

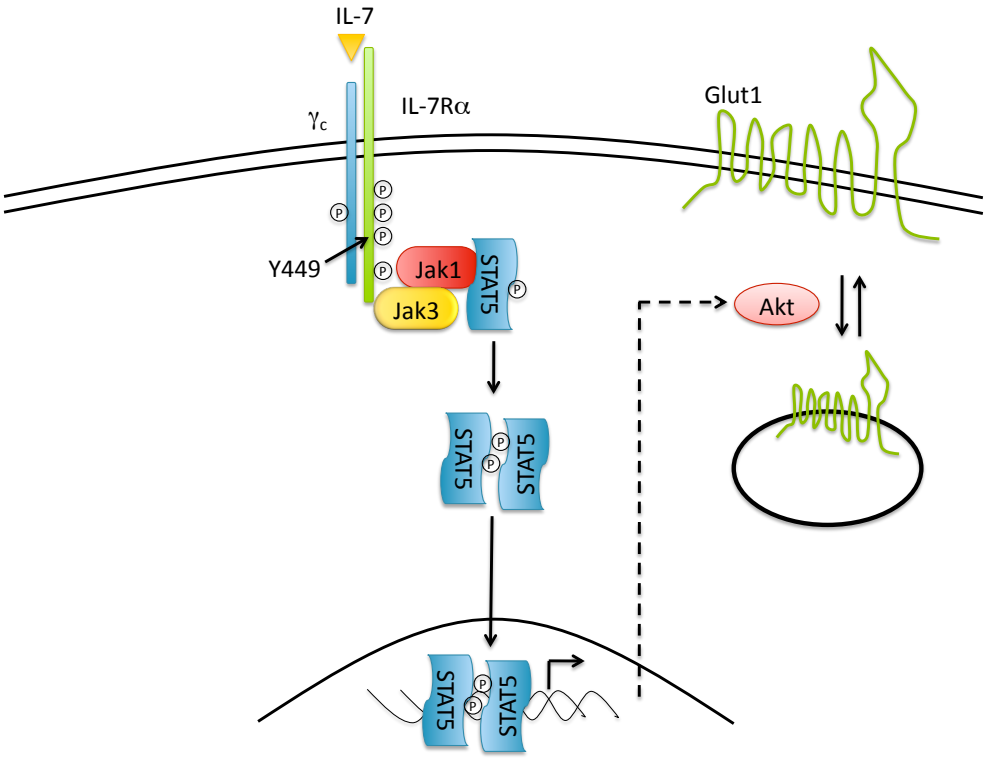

Figure 1.3: IL-7R signaling

The IL-7R is comprised of the common γ_c and the IL-7Rα chain. Phosphorylation of Y449 is critical for signaling to Jak1 and Jak3, which activate the transcription factor STAT5. STAT5 transcriptionally activates Akt, which can in turn increase Glut1 surface levels.

dramatic decrease in T cell numbers similar to that of IL-7R$^{-/-}$ or JAK3$^{-/-}$ animals (Yao et al., 2006). When only one STAT5 isoform is lost, is seems possible that the other isoform is able to compensate as neither STAT5a nor STAT5b knockout resulted in an effect on T cell development or survival (Teglund et al., 1998). The amino acid Y449 on the IL-7R is required for activation of the Jak/STAT signal transduction pathway and for induction of the PI3K/Akt signaling cascade (Osborne et al., 2007; Pallard et al., 1999). Recent evidence indicates that STAT5 transcriptionally initiates a pathway to activate Akt and that both signals are necessary for IL-7 to promote regulation of glucose usage in primary T cells (Wofford et al., 2008) and in T-ALL cells (Barata et al., 2004). STAT5 also induces Pim kinases, and ectopic expression of Pim1 can rescue thymic development in IL-7 and γ_c knockout animals (Jacobs et al., 1999). Pim kinases have also been shown to regulate glucose metabolism (Fox et al., 2003). The ability of IL-7 signaling to regulate glucose uptake has been implicated in regulating T cell survival (Barata et al., 2004; Rathmell et al., 2001; Wofford et al., 2008).

1.4.3 Regulation of T cell survival

The critical role of IL-7 at multiple stages in T cell development demonstrate that IL-7 acts as a unique survival signal. However, the mechanisms downstream of the receptor that prevent cell death remain clouded. Regulation of Bcl-2 family members by IL-7 has emerged as candidate for regulation of cell survival, but as will be discussed below, may not be the only regulating factor, and glucose metabolism may play a key role.

1.4.3.1 IL-7 mediated regulation of glucose metabolism

IL-7 activates multiple signals known to regulate glucose metabolism such as STAT5 and the PI3K/Akt pathways. IL-7 may mediate regulation of glucose metabolism

through Akt, which is known to increase the amount of Glut1 on the cell surface, and consequently to alter the rate of glucose uptake into the cell (Wieman et al., 2007). Correspondingly, IL-7 cytokine dependent T cell lines, when withdrawn from IL-7, decrease glucose uptake (Khaled and Durum, 2003). Similarly, primary T cells experience a decrease in glucose uptake *in vitro*, and this reduction can be partially rescued by IL-7 (Rathmell et al., 2001; Wofford et al., 2008). Removal from cell extrinsic signals by culturing T cells also results in a decrease in glycolytic flux, which can be fully maintained by addition of IL-7 (Rathmell et al., 2001). It has been shown that regulation of glucose metabolism is required for maximal IL-7 mediated survival *in vitro* and suggested that the energy that becomes available within the cell due to IL-7 signaling is necessary to continue life (Wofford et al., 2008). However, the ability of IL-7 to regulate glucose metabolism *in vivo* and the role of this regulation has not been assessed. Although IL-7 dependent regulation of Bcl-2 family members has been implicated in the ability of the cytokine to maintain cell survival, this is not likely to be the only pathway for preventing apoptosis as IL-7 can inhibit T cell death in Bcl-2 deficient cells (Nakayama et al., 1995), and suggests that metabolism downstream of IL-7 may play a role.

1.4.3.2 IL-7 and Bcl-2 family members

Although IL-7 regulation of glucose metabolism may be a critical mediator of cell survival, most analyses of the anti-apoptotic effects of IL-7 have centered on the Bcl-2 family of cell survival controlling proteins. Bcl-2 was the first member of its family identified, when mis-expression of the Bcl-2 gene was found in human B cell lymphomas and Bcl-2 was later characterized as a potent anti-apoptotic protein (Strasser et al., 1996). IL-7 was found to regulate Bcl-2 protein levels during a T cell lineage developmental transition point (Rathmell et al., 2001; von Freeden-Jeffry et al., 1997). In

early studies, transgenic expression of Bcl-2 resulted in increased survival of T cells, but did not increase T cell numbers (Sentman et al., 1991; Strasser et al., 1991). However, it was later suggested that the lack of increase in cell numbers indicative of alteration in a homeostatic regulatory protein may be due to Bcl-2's anti-proliferative effect (Janumyan et al., 2003). Conversely, genetic ablation of Bcl-2 produced mice that are born with normal numbers of T cells, but this level declines rapidly after 1 month, suggesting that Bcl-2 may not be necessary during development (Veis et al., 1993). Bcl-2 has, however, been shown to be regulated by IL-7 in T cell lines (Hernandez-Caselles et al., 1995; Lee et al., 1996). Withdrawal of IL-7 from pro-T cells resulted in decreased Bcl-2 levels and re-addition of IL-7 in culture could only partially rescue Bcl-2 levels (Kim et al., 1998). In mice lacking the γ_c, over expression of Bcl-2 rescued T cell development, but not that of B cells or NK cells (Kondo et al., 1997a). Multiple studies have assayed the ability of over expression of Bcl-2 to compensate for T cell homeostasis signals. Bcl-2 over expression in an IL-7R$^{-/-}$ background resulted in partial rescue of T cell development, numbers, and function (Akashi et al., 1997; Maraskovsky et al., 1997). Although Bcl-2 expression was able to rescue T development, it could not fully substitute for IL-7 signals. For example, γδ T cell development remained impaired (Nakajima and Leonard, 1999), and IL-7 can inhibit T cell death even in Bcl-2 deficient cells (Nakayama et al., 1995).

A second anti-apoptotic protein, Bcl-xL, has also been implicated in the regulation of T cell survival. While Bcl-xL levels decrease just prior to death of activated T cells and ectopic expression of Bcl-xL prevents cell death (Broome et al., 1995), the expression patterns of Bcl-xL in developing thymocytes do not coincide with IL-7R levels. That, and the discovery that Bcl-xL is expressed at very low levels in resting T cells (Noel et al., 1996), indicated that while Bcl-xL may regulate survival, it is not likely to be under control of IL-7 signaling.

The anti-apoptotic family member Mcl-1 is also expressed and is critical in naive T cells and may play a role in IL-7 regulation of cell survival. Over expression of Mcl-1 in hematopoietic precursor cells resulted in increased cell viability of B and T cells, but did not result in overall changes in the number of these cells (Zhou et al., 1998). Over expression of Mcl-1 did however ultimately lead to increased immortalization and prevalence of B cell lymphoma (Zhou et al., 2001). These data suggested that while Mcl-1 was capable of maintaining cell survival, it was not sufficient as a homeostatic factor to greatly increase cell numbers. As it was not possible to produce Mcl-1 null animals due to peri-implantation lethality (Rinkenberger et al., 2000), conditional Mcl-1 knockout animals were produced. Loss of Mcl-1 in peripheral T cells resulted in a rapid decline in T cell numbers following excision of the *mcl-1* gene, and more importantly, the survival advantage imparted by IL-7 *in vitro* required the expression of Mcl-1 (Opferman et al., 2003). Thus Mcl-1 is essential for T cell survival and the anti-apoptotic effects of IL-7.

Pro-apoptotic family members such as Bad, Bim, Bax, and Bak have also been implicated in the regulation of T cell survival, although not as directly as Bcl-2 or Mcl-1. Bad may be involved in cell death following withdrawal from IL-7 in a cytokine dependent cell line, but this role has not been fully examined in primary cells (Li et al., 2004) and Bad-deficient mice are grossly normal until late in life, when lymphomas may develop (Ranger et al., 2003). Loss of Bax initially restored thymic cellularity of IL-7R$^{-/-}$ animals, but after one month cell numbers returned to IL-7R$^{-/-}$ levels (Khaled et al., 2002). Bax/Bak double knockout animals displayed increased peripheral lymphoid cells, but this was due to alterations in thymic output and enhanced survival of memory cells more so than a direct effect on naive T cell homeostasis (Rathmell et al., 2002). Loss of Bim significantly restored peripheral T cell survival in IL-7R$^{-/-}$ animals (Pellegrini et al., 2004). The role of Bim in IL-7 mediated cell survival has been further clarified in a model

where $Bim^{+/-}$ $Bcl\text{-}2^{-/-}$ animals were injected with anti-IL-7 antibodies and found that cell survival was decreased compared to T cells in wildtype animals (Wojciechowski et al., 2007). Additionally, $Bim^{+/-}$ $Bcl\text{-}2^{-/-}$ T cells were refractory to increased IL-7 mediated survival *in vitro* (Bouillet et al., 2001). The ratio of these pro- and anti-apoptotic proteins was altered by IL-7 and is critical to control cell survival. It is clear that IL-7 signaling mediates cell survival at least in part through regulation of Bcl-2 family members, but this does not explain all functions of IL-7 and does not exclude involvement of glucose metabolism. It has been previously demonstrated in hematopoietic cell lines that increased glucose metabolism confers a survival advantage by maintaining Mcl-1 levels and glucose withdrawal results in induction of Puma, a pro-apoptotic Bcl-2 family member (Zhao et al., 2007; Zhao et al., 2008). Together these data suggest that IL-7 may regulate T cell homeostasis in part through glucose metabolism control of Bcl-2 family members.

1.5 Questions to be addressed

This work focuses on the role of glucose metabolism in T cell function, which will be addressed in a two-fold approach. First, the role of glucose metabolism in a T cell immune response will be examined. T cells are known to upregulate glucose uptake upon stimulation, but it is not clear if this increase in uptake is required for T cell function. Can T cells efficiently elicit an immune response in the absence of glucose, but with other carbon fuels plentiful? Conversely, I also address if glucose uptake is limiting for T cell activation by determining if increased glucose uptake results in an elevated ability of T cells to perform their function. In addition to studying the role of glucose uptake during T cell activation, the signaling pathways that control this upregulation of

glucose uptake will be examined. While many pathways are triggered following TCR stimulation, which signals regulate glucose uptake will be investigated and the relative contribution of the TCR and costimulatory signals to mediate this regulation will be determined.

Although it is clear that glucose uptake increases dramatically during T cell activation, there is only circumstantial evidence to indicate that glucose metabolism plays a role in naive T cells. All cells, even quiescent naive T cells, require a basal level of energy to survive, and loss of energy available from carbon sources may lead to cell death. To investigate the importance of glucose metabolism in naive T cell survival and homeostasis, the cell exogenous signal interleukin 7 will be examined for its ability to regulate glucose metabolism. The production of an inducible knockout model for IL-7R will allow for novel study of the *in vivo* importance of IL-7 in the regulation of mature T cell homeostasis and determine if this regulation is mediated in part through control of glucose metabolism.

2. Materials and Methods

2.1 Mice

Glut1 and mAkt transgenic mice have been previously described (Rathmell et al., 2003a; Zhao et al., 2007) and were bred and maintained on the C57Bl6/J background (Jackson Laboratory, Bar Harbor, ME). Double transgenic mice were produced at Duke University through selective breeding. $Akt1^{-/-}$ mice were a generous gift from Morris Birnbaum, University of Pennsylvania, PA. IL-$7R^{flox}$ transgenic mice were produced from murine cDNA of IL-7Rα and cloned into the pLck.E2 vector with flanking *loxP* sequences and micro-injected into C57Bl6/J oocytes by the Duke Transgenic Mouse Facility. Cre:ER transgenic animals expressed this transgene under a Ubiquitin promoter and were a generous gift from Eric Brown at the University of Pennsylvania, PA (Ruzankina et al., 2007). To excise the IL-$7R^{flox}$ transgene, mice were treated with 0.15mg Tamoxifen (Sigma, St. Louis, MO) dissolved in corn oil by intraperitoneal injection for two consecutive days. Animals were sacrificed three days after the first treatment. Mice were housed and cared for at Duke University and appropriate institutional boards approved all animal procedures. Unless otherwise indicated, experiments were performed on mice between 6 and 8 weeks of age.

2.2 T cell purification and culture

T cells were purified via negative selection from spleen and mesenteric lymph nodes unless otherwise specified (StemSep, Vancouver, BC, Canada) and cultured in RPMI 1640 (Mediatech, Inc., Herndon, VA) supplemented with 10% Fetal Bovine Serum (Gemini Bio-Products, Woodland, CA). Glucose free RPMI 1640 (Invitrogen, Carlsbad,

CA) with 10% dialyzed FBS (Gemini Bio-Products) was used for culture of cells in limited glucose, and glucose (Sigma) was filter sterilized before being added to cultures to indicated concentrations. Following purification, T cell stimulation was achieved by culture of T cells on plates coated with anti-CD3ε (clone 145-2C11) at various doses and anti-CD28 (clone 37.51) (both from BD Pharmingen, San Diego, CA) at 5μg/mL in PBS. Where indicated, IL-7 was supplemented into media at a concentration of 10ng/mL (eBioscience, San Diego, CA). Cells were counted and cell size was determined on a Coulter Z2 particle counter (Beckman Coulter, Miami, FL).

In some cases, T cells were treated with small molecule inhibitors. The drug LY294002 (Calbiochem, San Diego, CA) was dissolved in DMSO, and cells were treated with 10μM to inhibit PI3K. Cyclosporine A (CsA) (Sigma) was added to culture at a final concentration of 0.5μg/mL to block calcineurin. U0126 (Enzo Life Sciences International, Inc., Plymouth Meeting, PA) was used at 10μM to block Mek1/2- MAPK mediated signaling and GF 109203X (Gf) (Calbiochem, Gibbstown, NJ) at 10μM was added to cultures to block PKC signaling.

2.3 Macrophage co-cultures

Macrophages were derived from murine bone marrow cultured in RPMI with addition of 3ng/mL GM-CSF (Peprotech, Rocky Hill, NJ) for 7 days. Bone marrow cultures were washed and macrophages were trypsinized, re-plated, and treated with LPS from E. coli O111:B4 (Sigma) at 100ng/mL and CTLA4-Ig (BD Pharmingen) at 1μg/mL or anti-ICAM-1 (BD Pharmingen) at 2μg/mL where indicated for 1 hour prior to addition of T cells. T cell macrophage co-cultures were cultured with or without addition of indicated doses of anti-CD3 (BD Pharmingen) at various doses for 1 day. To measure glucose uptake, non-adherent cells were removed and re-plated for one hour.

Cells that remained non-adherent were >90% T cells and were subject to glucose uptake analysis.

2.4 Proliferation, survival and flow cytometry

Proliferation was determined by staining T cells with 5,6-carboxyfluorescein succinimidyl diester (CFSE) (Molecular Probes, Eugene, OR) prior to culture. This dye binds to proteins within the cell and is diluted by each cellular division allowing for quantification of proliferation by flow cytometry. Survival assays were performed via Propidium Iodide (Molecular Probes) exclusion and flow cytometry. T cells were stained with fluorescently conjugated antibodies against murine CD4, CD8, CD25, CD44, CD69, Thy1.1, and Thy1.2 (BD Pharmingen). IL-7R levels were analyzed with anti-IL-7R conjugated to biotin (clone A7R34 from eBioscience) and a Steptavadin PE-Cy5 conjugated secondary (eBioscience) followed by flow cytometry. Surface Myc-Glut1 was detected with anti-Myc (4A6, Upstate, Lake Placid, NY) followed by PE anti-mouse (BD Pharmingen). Glut1 total protein levels were determined by fixation in 1% paraformaldehyde for ten minutes at 37°C, permabilization with 100% methanol on ice for 30 minutes, and anti-Glut1 antibody (Abcam, Cambridge, MA), followed by a fluorescently conjugated anti-rabbit secondary and flow cytometry. Flow cytometry was performed on a FACscan or FACSCanto (Becton Dickinson, San Jose, CA) and analyzed with FlowJo software (Tree Star Inc., Ashland, OR).

2.5 ELISA

Enzyme-linked immunosorbent assays (ELISAs) were performed on supernatant from T cell cultures using rat antibodies against murine IL-2 and IFNγ (BD Pharmingen),

as previously described (Rathmell et al., 2003a). In some cases IL-2 ELISA was performed using Endogen® Mouse IL-2 ELISA Kit (Pierce Biotechnology, Rockford, IL) as per the manufacturer's instructions. Blood immunoglobulin isotype levels were determined on blood obtained via cardiac puncture through the use of Mouse Immunoglobulin Isotyping ELISA kit (BD Pharmingen) per manufacturer's instructions.

2.7 Immunoblotting

To probe for Glut1, cells were lysed for western blotting for one hour on ice in PBS plus 1% Triton X-100 and 0.1% SDS containing protease inhibitors (BD Pharmingen), as previously described (Wieman et al., 2007). In some cases, lysates were treated with PNGase F (New England Biolabs, Ipswich, MA) to remove glycosylation, as per manufacturer's instructions. Briefly, proteins were denatured for 30 minutes at room temperature, followed by incubation with NP40, G7, and PNGase F for 1 hour at 37°C. When probing for any other protein, cells were lysed in radioimmunoprecipitation assay buffer as previously described (Zhao et al., 2007). Equivalent protein concentrations were loaded on SDS-PAGE gels (Bio-Rad, Hercules, CA) and probed with primary antibodies: mouse anti-Akt1, rabbit anti-phospho -Akt (S473), rabbit anti-phospho-STAT5 (Y694) (Cell Signaling, Danvers, MA), rabbit anti-Bcl-2, mouse anti-STAT5 (BD Pharmingen), rabbit anti-Mcl-1 (Biolegend, San Diego, CA), rabbit anti-Glut1 (Abcam, Cambridge, MA), mouse anti-Pim1 (Santa Cruz, Santa Cruz, CA), or mouse anti-actin (Sigma). Secondary antibodies anti-mouse HRP (BD Pharmingen) and anti-rabbit HRP (Cell Signaling) were followed by ECL-Plus (Amersham Biosciences, Piscataway, NJ) for visualization. Secondary antibodies Alexa Fluor 680 anti-rabbit IgG (Invitrogen) and IRDye 800 anti-mouse IgG (Li-Cor Biosciences, Lincoln, NE) were detected using a

Li-Cor Odyssey infrared detection system (Li-Cor Biosciences). Contrast and brightness were adjusted uniformly for each image.

2.8 Microscopy

Kidneys were embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA), and flash frozen. 12μm sections were cut, washed in PBS, fixed in 1% Paraformaldehyde, blocked with 2% FBS in PBS, and stained with FITC anti-Mouse IgG (BD Pharmingen). Sections were then washed and mounted with Fluoromount-G (SouthernBiotech, Birmingham, AL) and examined microscopically at 200X total magnification with a Zeiss Axio Imager upright wide field fluorescence microscope and Metamorph software (Universal Imaging Corporation, Downingtown, PA).

2.9 Glucose uptake

Glucose uptake was performed as previously described with minor modification (Rathmell et al., 2003b; Wieman et al., 2007). Briefly, cells were suspended in Kreb's Ringer HEPES and 2-deoxy-D-[H^3]glucose (2μCi/rxn) was added for 10 minutes at 37°C. The reactions were quenched with ice-cold 200μM phloretin (Calbiochem) and washed to separate cells from remaining radioactivity. Cells were solubilized with 1M NaOH prior to measurement of radioactivity transported into cells with a scintillation counter.

2.10 Glycolysis

Glycolytic flux analysis was also performed as previously described (Rathmell et al., 2001). Briefly, freshly isolated 2 X 10^6 viable T cells were washed in PBS and incubated in glucose-free Kreb's buffer for half an hour prior to addition of 10μCi of

D-[5-^{3}H](N)-glucose (Perkin Elmer, Waltham, MA) and addition of non-radio-labeled glucose to bring total glucose concentration to 10mM prior to culture for 1 hour. Reaction was stopped by addition of an equal volume of 0.2N HCl. [^{3}H]H_20 was separated from [^{3}H]-Glucose by evaporated equilibrium in a sealed environment. Levels of [^{3}H]-H_20 produced during glycolysis were measured on a scintillation counter.

2.11 Hexokinase activity

Hexokinase activity was determined as previously described (Rathmell et al., 2003b). Briefly, glucose-6-phosphate production was coupled to the production of NADPH, levels of which could be determined by spectrophotometric reading at 340nm. Equivalent live cell numbers were lysed in 0.1% Trition X-100 in PBS and combined with 3mM glucose, NADP (5mg/mL), glucose-6-phosphate dehydrogenase (100U/mL), 220mM ATP, and 1% monothiolglycerol. Absorbance was read at 30 second intervals for 30 minutes on a SpectraMax Plus384 (Molecular Devices, Sunnyvale, CA).

2.12 Quantitative real time PCR analysis

RNA was isolated and quantitative real time PCR was performed and normalized as previously described (Altman et al., 2009). Briefly, cells were lysed in Trizol (Invitrogen) and mRNA was isolated by chloroform extraction and ethanol precipitation. RNA was treated with DNAase (Invitrogen) and reverse-transcribed using the Super Script II Reverse Transcriptase (Invitrogen). PCR was performed using IQ Sybr-Green Supermix (Bio-Rad) on a Bio-Rad ICyler Real Time PCR machine. The primers used for Glut1 were: CGGGTATCAATGCTGTGTTC and

GTCCAGCTCGCTCTACAACA and were normalized to RT-PCR amplification of β2-microglobulin (β2M): ACCGGCCTGTATGCTATCCAGAAA, GGTGAATTCAGTGTGAGCCAGGAT.

2.13 Bone marrow reconstitution

Bone marrow reconstitutions were performed similarly to as previously described (Pui et al., 1999). Briefly, C57BL/6J mice were injected with 5mg Fluorouracil (American Pharmaceutical Partners, Inc, Schauburg, IL) four days prior to isolation of whole bone marrow. Bone marrow was cultured in DMEM (Invitrogen) with Pen Strep, Glutamine, 15% FBS, 5% Wehi-3B conditioned media, 10ng/mL IL-3 (eBioscience), 50ng/mL Stem Cell Factor (Peprotech), and 10ng/mL IL-6 (Peprotech). Cells were cultured for 1 day prior to infection with MSCV-Myc-Glut1 (Myc-Glut1 was a generous gift of Robert Farese, University of Southern Florida, Tampa, FL). Cells were cultured for an additional day, washed, resuspended in PBS, and injected via tail vein into C57Bl6/J $RAG^{-/-}$ (Jackson Laboratory) mice that were lethally irradiated the previous day. Cells were allowed to reconstitute mice for two months prior to analysis.

3. Glucose Uptake is Limiting in T Cell Activation and Requires CD28-Mediated Akt Dependent and Independent Pathways

This chapter appears in modified form in:

Journal of Immunology 180: 4476-4486 (2008)

Copyright 2008. The American Association of Immunologists, Inc.

Catherine E. Herman, Nancie J. MacIver, Jessica A. Wofford, Heather L. Wieman, Jeremy J. Hammen, and Jeffrey C. Rathmell

3.1 Introduction

A functional immune response requires rapid and extensive cell growth, proliferation, and production of effector proteins, such as cytokines. To perform these functions, the metabolic and biosynthetic demands of lymphocytes become dramatically increased after activation (Frauwirth and Thompson, 2004; Krauss et al., 2001). When resting, T lymphocytes meet basal energy demands primarily through mixed usage of glucose and glutamine (Bental and Deutsch, 1993). After activation, glucose metabolism increases as a source of energy and cellular biosynthesis (Bental and Deutsch, 1993; Frauwirth et al., 2002; Frauwirth and Thompson, 2004; Greiner et al., 1994). While some regulation of cellular metabolism is anapleurotic and is met by enhanced flux due to relief of feedback inhibition in pathways whose end products are rapidly utilized, it has become increasingly apparent that regulatory mechanisms also exist to promote specific metabolic pathways and phenotypes (Fox et al., 2005; Frauwirth and Thompson, 2004; Plas and Thompson, 2005). In lymphocytes, control of nutrient uptake by cell extrinsic signals has been proposed as a key point of regulation for cellular metabolism (Rathmell et al., 2000). If appropriate signals are not received, nutrient uptake decreases to a level

below that capable of supporting cellular demands, leading to inhibition of cell proliferation and ultimately, apoptosis (Ciofani and Zuniga-Pflucker, 2005; Rathmell et al., 2001; Rathmell et al., 2000). In particular, signals and signaling pathways that control lymphocyte glucose uptake may be critical to cell function, viability, and immune response. Insufficient glucose can lead to deficient responses of activated T cells (Cham and Gajewski, 2005) and induction and activation of pro-apoptotic Bcl-2 family proteins such as Noxa and Bax (Alves et al., 2006; Chi et al., 2000). However, the importance of elevated glucose uptake in T cell function and the signaling pathways that are required to promote increased glucose uptake in T cell activation are uncertain.

T cell activation and costimulation may regulate glucose transport in lymphocytes via control of glucose transporter expression, localization, and function. The primary glucose transporter in hematopoietic cells, Glut1, is expressed at low levels in resting T cells and is upregulated upon T cell activation (Chakrabarti et al., 1994; Frauwirth et al., 2002; Frauwirth and Thompson, 2004). Increased Glut1 levels and glucose uptake correlate with increased cellular growth and proliferation, such as in thymocytes, where increased Glut1 expression is highest in proliferating cells (Swainson et al., 2005; Yu et al., 2003). Control of Glut1 trafficking and activity are also key elements regulating glucose uptake. Similar to the insulin-responsive glucose transporter, Glut4, Glut1 cell surface localization is controlled by extrinsic signals (Wieman et al., 2007). Among signaling pathways initiated in T cell activation, the phosphatidyl-inositol 3-kinase (PI3K)/Akt pathway has been shown to promote both Glut1 cell surface trafficking and activity (Edinger and Thompson, 2002; Kan et al., 1994; Rathmell et al., 2003b; Wieman et al., 2007). In the absence of these signals, Glut1 remains intracellular and may be degraded in lysosomes to restrict glucose uptake (Edinger and Thompson, 2002; Wieman et al., 2007).

In resting T cells, glucose metabolism is maintained by cytokines such as IL-7 (Rathmell et al., 2001), while T cell activation increases glucose metabolism and supports T cell proliferation through the ligation of the TCR/CD3 and a second costimulatory signal (Frauwirth and Thompson, 2004). In particular, CD28 and its ligands B7.1 and B7.2 are sufficient to augment CD3 signals and promote glucose metabolism (Frauwirth et al., 2002). Mechanistically, costimulation may promote glucose metabolism by enhancing antigen receptor initiated signaling pathways, such as NFAT, NF-kB, MAPK, and Ras activation. Costimulation may also preferentially activate specific signaling pathways, such as CD28 activation of the PI3K/Akt pathway, to promote glucose uptake and metabolism. Activation of PI3K may be a particularly important regulator of glucose uptake, as PI3K inhibition prevented increased glucose metabolism after lymphocyte activation or cytokine stimulation, and decreased glucose uptake of leukemic cells (Barata et al., 2001; Bentley et al., 2003; Doughty et al., 2006; Frauwirth et al., 2002). Conversely, in addition to its ability to cause autoimmunity and lymphoma, expression of constitutively active Akt promoted increased glucose uptake and consumption in cell lines (Doughty et al., 2006; Rathmell et al., 2003b) and elevated glycolysis in primary T cells (Rathmell et al., 2003a). The PI3K/Akt signaling pathway may, therefore, play an important role in regulating the immune response and lymphocyte homeostasis through the regulation of glucose metabolism.

The role of glucose uptake in activation of naive T cells and the costimulatory signaling pathways necessary to promote it are not clear. Here it is demonstrated that glucose uptake is limiting in T cell activation, and elevated Glut1 expression enhanced T cell activation and led to increased serum immunoglobulin levels and immunoglobulin deposition in kidneys of aged mice. CD28 costimulation is required to maximally increase glucose uptake through distinct signaling pathways. With low TCR signal

strength, CD28 increased Glut1 expression, possibly by augmenting TCR-induced signals. In contrast, CD28 signaling through the Akt pathway was insufficient to upregulate Glut1 protein, yet increased glucose uptake, possibly via enhanced cell surface Glut1 trafficking. Supporting dual mechanisms for CD28-mediated regulation of Glut1 protein levels and cell surface trafficking, transgenic expression of Glut1 together with constitutively active Akt synergistically increased glucose uptake. This diminished the requirement for CD28 costimulation, and led to rapid accumulation of activated T cells *in vivo*. These data show that expression of Glut1 and regulation of glucose uptake are controlled by CD28 through separable Akt-dependent and Akt-independent pathways and are limiting in T cell activation.

3.2 Results

3.2.1 Costimulation via CD28 is necessary for increased glucose uptake upon T cell activation

In TCR-stimulated human peripheral T cells, an increase in glucose uptake has been previously demonstrated upon induction of the CD28 costimulatory signal when provided by antibody-coated beads (Frauwirth et al., 2002). Consistent with this finding, glucose uptake in murine T cells increased dramatically at multiple doses of anti-CD3 antibody on coated plates in the presence of anti-CD28-mediated costimulation (Figure 3.1A). In contrast, T cells stimulated with only anti-CD3 increased glucose uptake modestly compared to CD28-costimulated cells. These data demonstrate that CD28 signaling can potently increase glucose uptake of TCR-stimulated T cells, but do not demonstrate if CD28 signaling is required among the myriad signals T cells may receive from antigen presenting cells to maximally increase glucose uptake. To determine the requirement for CD28 signals to induce glucose uptake, primary T cells

were cultured with bone marrow-derived, lipopolysaccaride (LPS)-activated macrophages to permit a broad range of antigen presenting cell-T cell interactions. Soluble anti-CD3 was added to cultures to bind F_c receptors on macrophages and induce TCR ligation. Costimulatory signals were intact or CD28 signals were inhibited by the

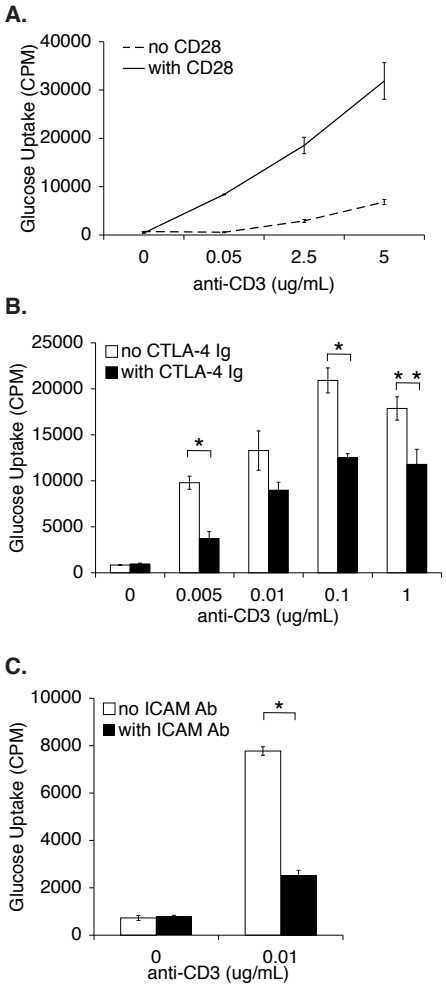

Figure 3.1: CD28 is required for maximal glucose uptake in T cell activation.

A. Purified T cells were stimulated on plates coated with or without anti-CD28 and with indicated doses of anti-CD3. Glucose uptake was measured after one day. **B, C.** T cells were co-cultured with LPS-stimulated bone marrow-derived macrophages. T cells were stimulated with anti-CD3 antibodies with and without (**B**) CTLA4-Ig or (**C**) anti-ICAM Antibody. After 1 day of co-culture, T cells were separated from macrophages and glucose uptake was analyzed (* indicates $p < 0.005$ and ** $p < 0.01$).

addition of Cytotoxic T Lymphocyte Antigen (CTLA-4) Ig, a solubilized receptor that blocks CD28 ligation. T cells recovered from culture with LPS-stimulated macrophages in the absence of anti-CD3 exhibited low levels of glucose uptake, demonstrating a requirement for TCR stimulation for increased glucose uptake (Figure 3.1B). If anti-CD3 was present in the culture, T cell glucose uptake was markedly increased after one day. Addition of CTLA-4 Ig to TCR-stimulated T cell/macrophage co-cultures even at high doses of anti-CD3, resulted in a significant decrease in T cell glucose uptake ($p < 0.01$) relative to cultures that did not receive CTLA4-Ig. Although CD28 is necessary for maximal increases in glucose uptake, it was not uniquely required, as blockade of ICAM signaling also reduced TCR-stimulated glucose uptake in macrophage co-cultures (Figure 3.1C).

3.2.2 Costimulation increases Glut1 protein levels and trafficking to the cell surface

Glucose uptake in lymphocytes is mediated largely by the glucose transporter, Glut1 (Chakrabarti et al., 1994; Rathmell et al., 2000). Changes in glucose uptake mediated by CD28 signaling may therefore be controlled through changes in message, protein expression, or surface levels of Glut1. T cell stimulation resulted in an increase in the level of glut1 message and a high concentration of anti-CD3 more than doubled glut1 message levels compared to low anti-CD3 (Figure 3.2A). Low anti-CD3 costimulation with CD28 resulted in a near five-fold increase in glut1 message compared to unstimulated cells. CD28 costimulation also increased glut1 mRNA in T cells stimulated with a high dose of anti-CD3, although this effect was much less pronounced. Similar to glut1 message, low levels of Glut1 protein were expressed in unstimulated T cells and T cells stimulated with low concentrations of anti-CD3 (Figure 3.2B). CD28 costimulation, however, elevated Glut1 protein levels in weakly anti-CD3 stimulated T

cells. With stronger anti-CD3 stimulation, Glut1 protein was induced in the absence of CD28 stimulation. CD28 costimulation may have additionally influenced Glut1 modification as Glut1 induced in the presence of CD28 demonstrated a lower mobility by SDS-PAGE at both anti-CD3 doses. This altered mobility may suggest enhanced Glut1 glycosylation due to altered protein trafficking (Edinger et al., 2003). To determine if this modification was due to elevated glycosylation, cell lysates were treated with the glycosylase, PNGase F. This treatment increased Glut1 mobility (Figure 3.2C) indicating that co-stimulation elevated both levels and glycosylation of Glut1 protein. Enhanced glycosylation may suggest altered Glut1 intracellular trafficking and has been shown to increase glucose uptake activity (Asano et al., 1991; Asano et al., 1993). Consistent with this regulation of Glut1 expression, blockade of CD28 signals on T cells stimulated by activated macrophages and anti-CD3 also showed that CD28 was necessary to maximally induce Glut1 in T cells receiving a weak TCR signal, but played only a modest role in Glut1 upregulation when a strong TCR signal was provided (Figure 3.2D).

In addition to expression, Glut1 trafficking to the cell surface must be controlled (Wieman et al., 2007) and regulation of glycosylation suggested CD28 may influence this signal. To investigate costimulation induced regulation of Glut1 surface levels, hematopoietic stem cells were retrovirally transduced to express a Myc-tagged Glut1, and used to reconstitute the immune systems of lymphopenic mice. This approach allowed for fixed Glut1 expression from an exogenous promoter and flow cytometric detection of cell surface Glut1 via the Myc tag in cells derived from infected HSCs and for determination of the role of CD28 in the regulation of Glut1 cell surface trafficking. T cells purified from reconstituted mice and stimulated with a low or a high dose of anti-CD3 with or without CD28 costimulation produced equivalent amounts of Myc-Glut1

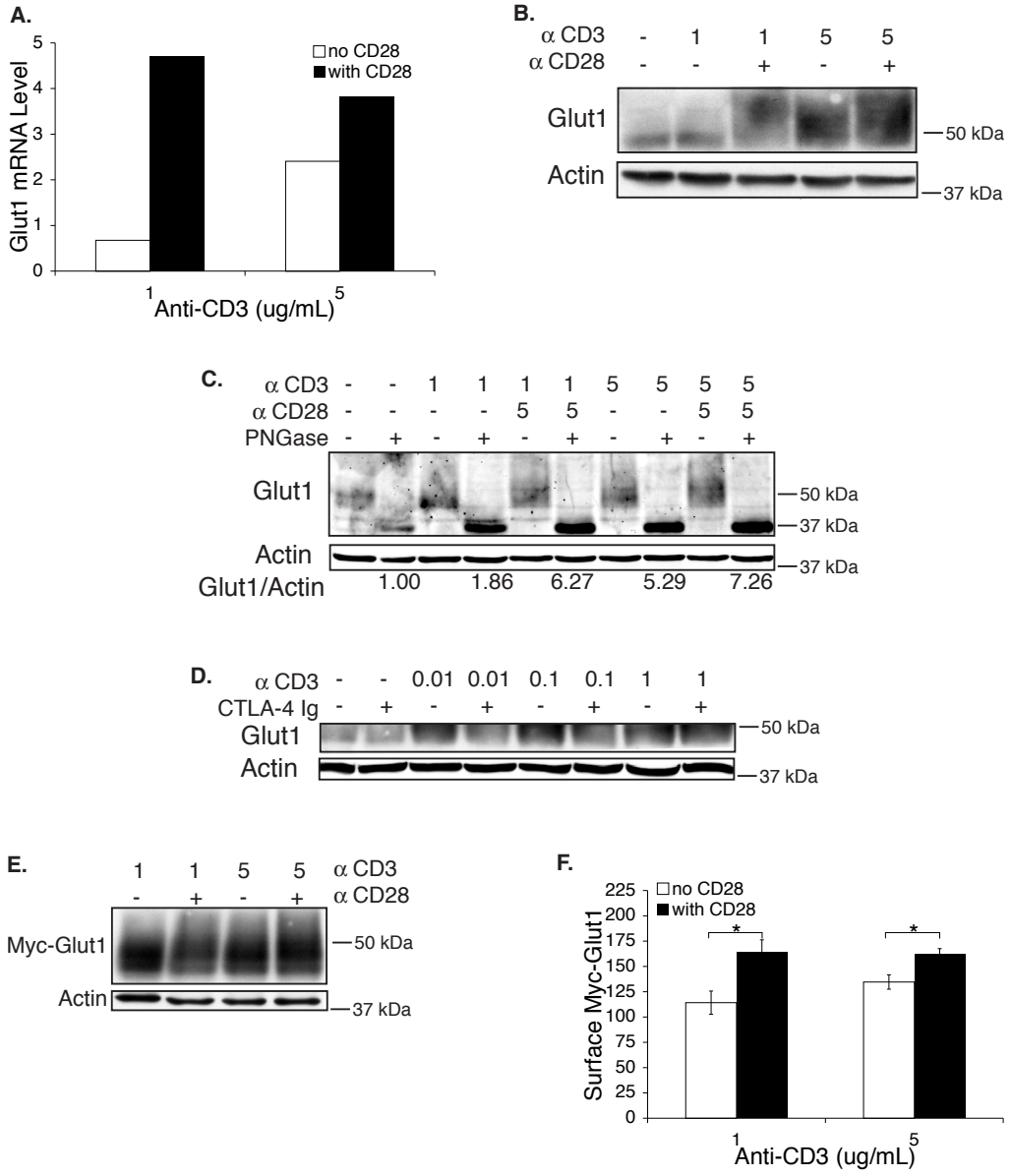

Figure 3.2: Costimulation induced Glut1 protein expression and trafficking to the cell surface.

A, B, C. Purified T cells were stimulated on plates coated with anti-CD3 and anti-CD28 antibodies at the indicated concentrations for 1 day prior to analysis and (**A**) RNA extraction and qRT-PCR analysis for Glut1 mRNA levels, (**B**) lysis and immunoblotting and (**C**) PNGase F treatement to de-glycosylate followed by immunoblot. Numbers indicate the quantification of the de-glycosylated Glut1 normalized to Actin control. **D.** T cells were co-cultured with LPS-stimulated bone marrow-derived macrophages and stimulated with anti-CD3 antibodies with and without CTLA4-Ig for one day prior to lysis and immunoblotting. **E, F.** T cells from mice reconstituted with hematopoietic stem cells infected with a Myc-tagged Glut1 were purified, stimulated on plates coated with the indicated dose of anti-CD3 with and without 5μg/mL anti-CD28 for 1 day and cells were (**E**) immunoblotted for Myc-Glut1 levels and (**F**) stained with anti-Myc to detect surface levels or Glut1 by flow cytometry. Means and standard deviations of five samples are shown (* indicates $p < 0.05$).

protein (Figure 3.2E). However, addition of the CD28 costimulation signal increased surface levels of Myc-Glut1 (Figure 3.2F). Due to over expression of exogenous Myc-Glut1, these results likely underestimate the role of CD28 on cell surface trafficking of endogenous Glut1. Together, these data indicate that costimultory signals are necessary to augment Glut1 expression in weakly TCR-stimulated cells and are necessary to promote maximal Glut1 cell surface levels regardless of TCR signal strength.

3.2.3 Glut 1 protein levels are regulated by multiple pathways

Both glucose uptake and Glut1 protein levels were regulated by TCR signaling, however, the pathways that may be involved in this control were unclear. As TCR signaling activates a variety of major cell signaling pathways such as MAPK, PKC, PI3K/Akt, calcium signaling, and NFκB, a candidate drug approach was utilized to assay the relative roles of multiple TCR signaling pathways. The drugs LY294006 (LY) and cyclosporine A (CsA), which inhibit PI3K/Akt and Ca^{2+}/calcineurin signaling respectively, were assessed for ability to affect glucose uptake, glut1 message, and Glut1 protein levels. Both drugs reduced the ability of anti-CD3 to induce glucose uptake, and CsA effectively reduced glucose uptake of costimulated T cells (Figure 3.3A). Glut1 message levels did not mimic this effect, as normalization to fresh cells of T cells stimulated with anti-CD3 alone in the presence of LY or CsA did not prohibit induction of glut1 message. However, both drugs prevented a further increase of message following costimulation (Figure 3.3B). The reduction in glucose uptake upon LY or CsA treatment may be due to effects on Glut1 protein levels. However, LY did not significantly affect Glut1 protein levels or mobility, and CsA treatment, surprisingly, may have resulted in an increase in Glut1 protein levels, although the mobility shift normally seen upon costimulation was reduced (Figure 3.3C). These data suggest that

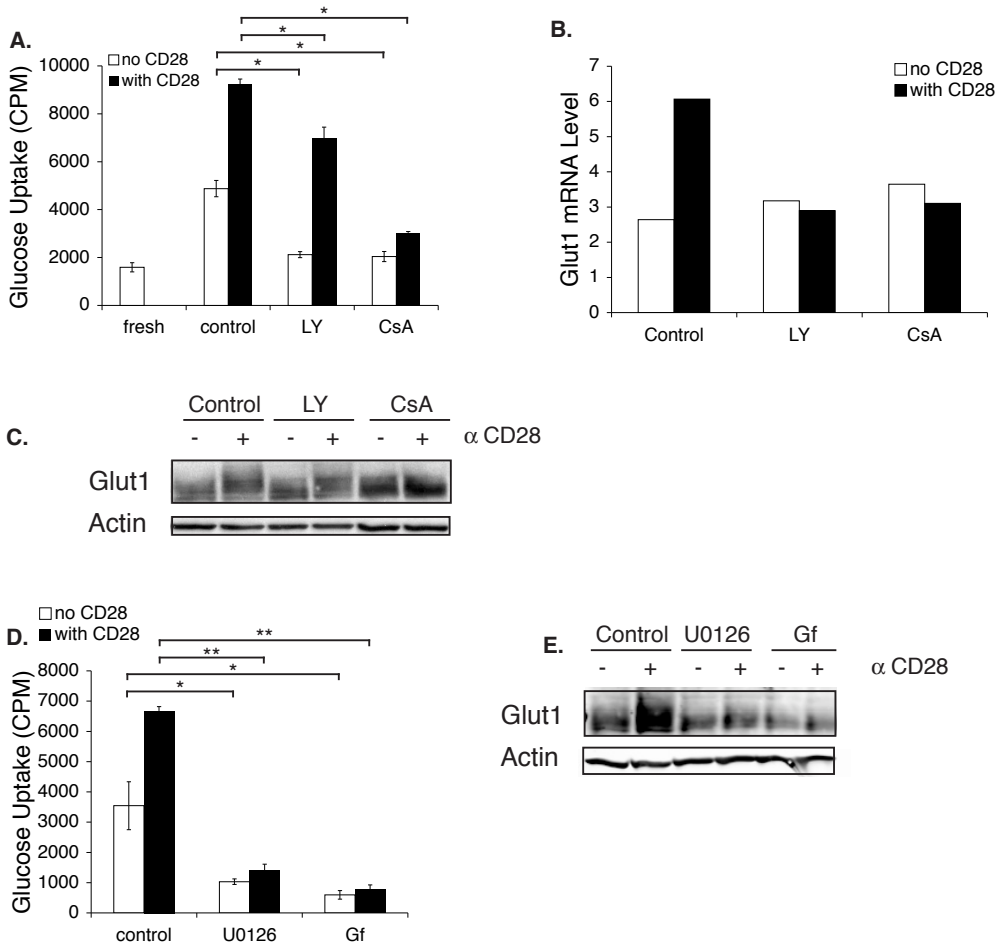

Figure 3.3: Multiple pathways regulate glucose uptake following T cell stimulation.

Purified T cells were pre-treated with 10μM LY294004 (LY) or 0.5μg/mL Cyclosporine A (CsA) for 30 minutes, then drug treatment was continued on plates coated with 5μg/mL anti-CD3 with or without 5μg/mL anti-CD28 for 18 hours and cells were analyzed (**A**) for ability to uptake glucose (*, $p < 0.01$), (**B**) Glut1 mRNA levels by qRT-PCR, and (**C**) total Glut1 protein levels following lysis and immunoblotting. **D.** and **E.** Purified T cells were pre-treated with 10μM U0126 or 10μM GF 109203X (Gf) for 30 minutes, then drug treatment was continued on plates coated with 1μg/mL anti-CD3 with or without 5μg/mL anti-CD28 for 15 hours and cells were analyzed (**D**) for ability to uptake glucose (*, $p < 0.05$; **, $p < 0.001$), and (**E**) total Glut1 protein levels following lysis and immunoblotting.

while the PI3K/Akt and Calcium signaling pathways are involved in regulation of glucose uptake, they may not mediate this through control of total cellular Glut1 protein levels.

To address other potential TCR signaling pathways, the drugs U0126 or GF109203X were applied to T cells during stimulation to inhibit the MAPK or PKC signaling pathways. Both U0126 and GF dramatically inhibited the increase in glucose uptake induced by anti-CD3 relative to control treated cells (Figure 3.3D). Additionally, costimulation was not able to rescue effects of these inhibitors, as glucose uptake remained low even in the presence of CD28 costimulation. This reduction in glucose uptake may have been due to changes in Glut1 protein levels as both U0126 and GF reduced Glut1 protein levels marginally with anti-CD3 stimulation and dramatically following CD28 costimulation (Figure 3.3E). This suggests that the MAPK and PKC pathways play an important role in the regulation of glucose uptake during T cell activation and may partially achieve this through the regulation of Glut1 protein levels. Further analysis of TCR signaling pathways will be required for exact determination of the factors necessary for increasing Glut1 protein levels.

3.2.4 Glucose is required for maximal immune response

T cells can utilize both glucose and glutamine as carbon sources in cellular metabolism (Bental and Deutsch, 1993; Greiner et al., 1994). Therefore, it was possible that elevated glucose metabolism may not be required for T cell growth and metabolism upon activation and other carbon sources may be sufficient to meet cellular demands. To determine the relative importance of glucose uptake in T cell survival and function during activation, freshly purified resting T cells were unstimulated or stimulated with anti-CD3 antibodies with or without anti-CD28 in media containing various concentrations of glucose at or below physiological levels that was supplemented with

2mM glutamine. Cells that received no stimulation survived poorly after two days regardless of glucose availability (Figure 3.4A). Stimulated T cells also failed to survive in the absence of glucose, but required only very low glucose concentrations to maintain viability. CD3-stimulated cells survived well at glucose levels as low as 0.05mM and addition of the CD28 costimulatory signal further increased survival. Similar to cell survival, T cells required glucose to produce IL2, but a very low glucose concentration was sufficient to elicit maximal IL2 secretion in T cells stimulated by anti-CD3 and anti-CD28 (Figure 3.4B). No IL2 was produced in unstimulated cells and very little IL2 was secreted by CD3 stimulated T cells in the absence of CD28 costimulation at any glucose concentration. In contrast to cell survival and IL2 production, T cell proliferation of stimulated cells was highly dependent on glucose availability (Figure 3.4C). T cells stained with CFSE and stimulated as described above with the addition of two different doses of anti-CD28 costimulation, were analyzed to determine the frequency of cells that divided twice or more. Nominal proliferation was detected in cells stimulated with CD3 alone; unstimulated cells did not proliferate regardless of glucose concentration. CD3-stimulated and CD28-costimulated T cells proliferated well in glucose concentrations of 0.5mM or higher, with cells exposed to higher levels of anti-CD28 proliferating more than those that received a lower dose of costimulation and despite continued IL2 production, failed to proliferate at lower glucose concentrations. Glucose availability also affected the ability of stimulated T cells to produce the inflammatory and Th1-promoting cytokine IFNγ. IFNγ was undetectable in media from unstimulated cells and low in CD3-only stimulated cells regardless of glucose concentration (Figure 3.4D). CD3-stimulated and CD28-costimulated cells produced IFNγ with cells exposed to a low dose of anti-CD28 producing only slightly more IFNγ than CD3 only stimulated cells, but

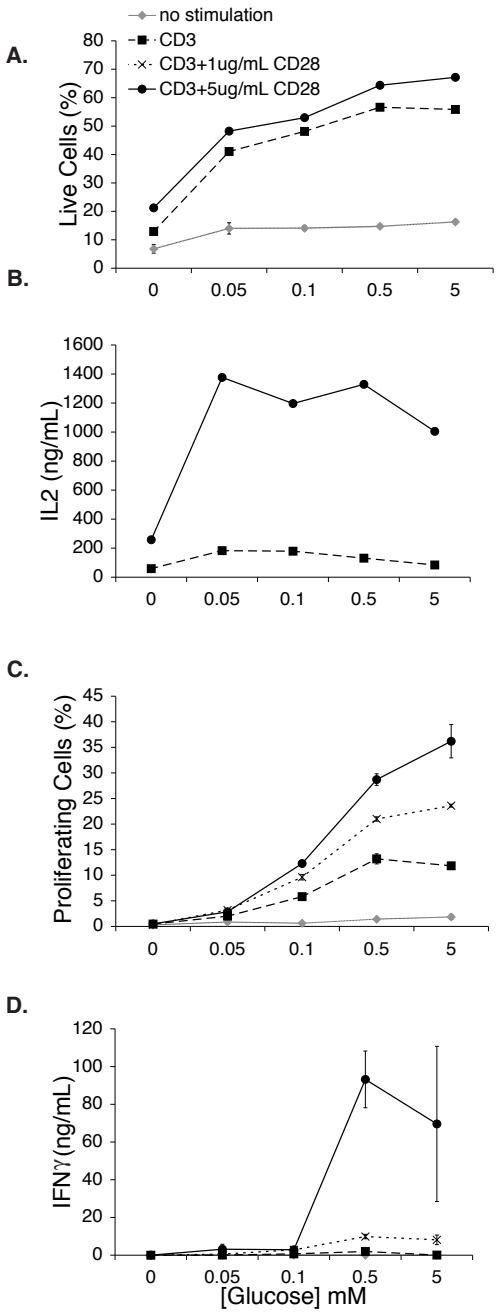

Figure 3.4: T cells require glucose for cell survival, IL-2 production, proliferation, and IFN-γ production.

Purified T cells were stained with CFSE, stimulated with 5μg/mL anti-CD3 with or without 1μg/mL or 5μg/mL anti-CD28 in glucose-free media supplemented with glucose to the indicated concentrations, and cultured for 2 days prior to analysis. **A.** Cell survival was determined by propidum iodide exclusion as analyzed by flow cytometry. **B.** IL2 production was assayed through ELISA on T cell supernatants. **C.** Reduction in CFSE staining was used to determine cell division by flow cytometry. **D.** IFNγ production was assayed through ELISA on T cell supernatants.

unlike the nearly glucose-independent regulation of IL2 secretion, IFNγ levels decreased with reduced glucose concentration. These data from naive T cell activation support the findings of Cham *et al*, who demonstrated with activated T cell blasts that IFNγ production is more sensitive to glucose availability than the production of IL2 (Cham and Gajewski, 2005). Therefore, regulation of glucose uptake is critical for proper immune function in both initial T cell activation and effector T cell populations.

3.2.5 Glut1 over-expression does not alter T cell development

Diminished glucose availability led to impaired T cell proliferation and IFNγ production following activation. To determine if glucose uptake is rate-limiting for proper T cell development, homeostasis, or activation, the role of increased glucose uptake capacity was analyzed in transgenic animals that overexpress Glut1 specifically in T cells (Zhao et al., 2007). Glut1 protein expression was elevated in both thymocytes and peripheral T cells from Glut1 transgenic animals compared to non-transgenic littermates (Figure 3.5A). Transgenic expression of Glut1 increased glucose uptake capacity compared to non-transgenic cells in both thymocytes and mature T cells, with a larger effect in peripheral T cells (Figure 3.5B). This increase in glucose uptake did not appear to alter T cell development, as percentages of thymic subpopulations and CD4 to CD8 ratios were normal in young mice (Figure 3.5C). Total thymocyte cell numbers and the number of splenic CD4 and CD8 T cells and B cells were also not significantly altered by Glut1 over-expression (data not shown, and Figure 3.5D). Further, Glut1 expression and its associated increase in glucose transport did not affect the activation phenotype of resting peripheral T cells. Flow cytometric analysis of CD25 and CD44 levels on CD4 and CD8 T cells showed little difference between Glut1 transgenic and non-transgenic littermates (Figure 3.5E). These data suggest that glucose uptake was not limiting in

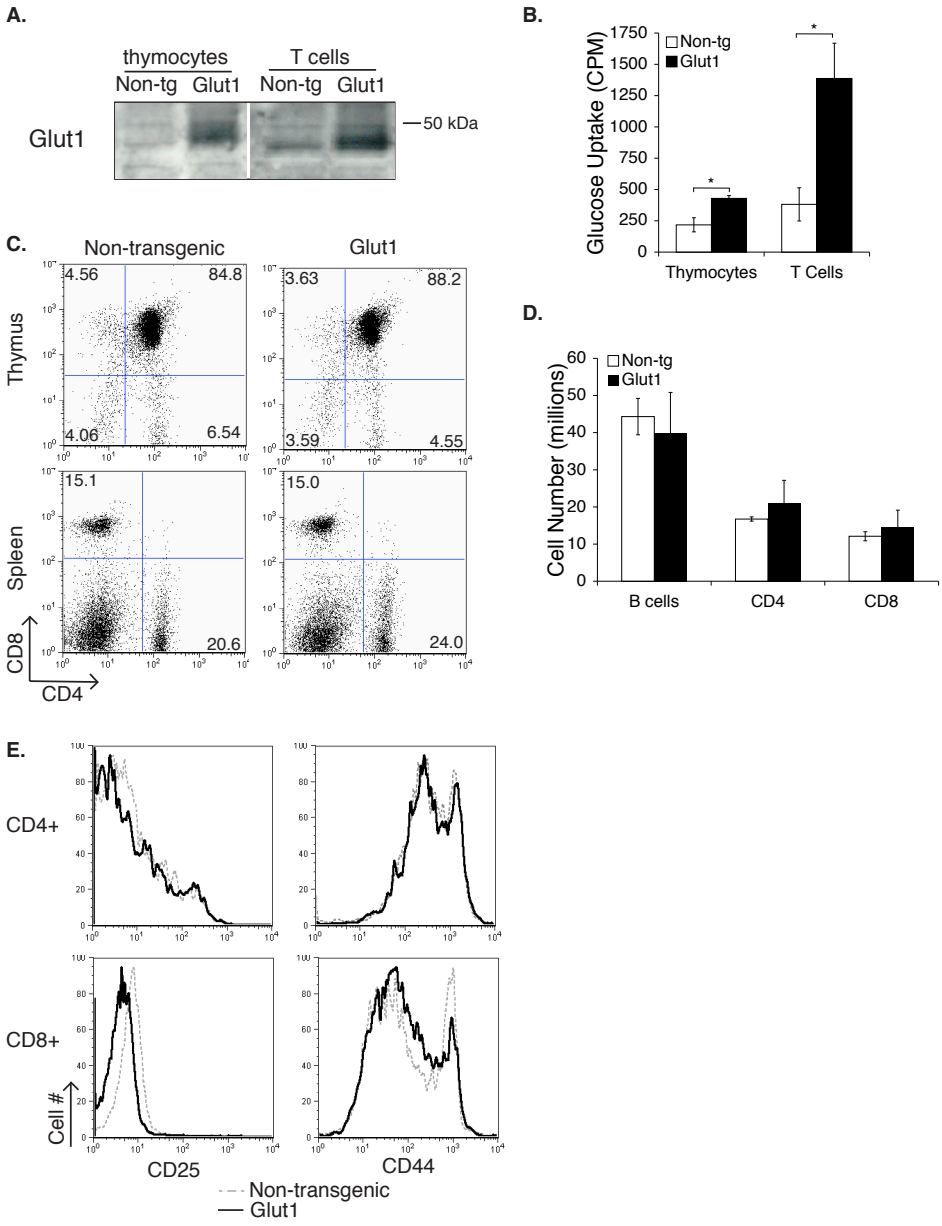

Figure 3.5: Glut1 over-expression in T cells does not alter T cell development or homeostasis in young mice.

A. Thymocytes and purified resting mature T cells from Glut1 transgenic and non-transgenic littermates were lysed and immunoblotted to determine Glut1 levels. **B.** Thymocytes and purified resting mature T cells from Glut1 transgenic animals were assayed for glucose uptake (*, $p < 0.005$). **C.** Thymocytes and whole spleen cell suspensions were stained with fluorescently conjugated antibodies against CD4 and CD8 and analyzed by flow cytometry. **D.** The number of cells in splenocyte suspensions was determined by particle size analyzer and multiplied by the percentage of whole spleen determined to be B cells, CD4+, or CD8+ cells as indicated by staining with B220, CD4, or CD8 fluorescently conjugated antibodies and flow cytometry. **E.** Levels of the activation markers CD25 and CD44 were determined in resting purified T cells from Glut1 transgenic and non-transgenic littermates by staining with fluorescent antibodies and flow cytometry.

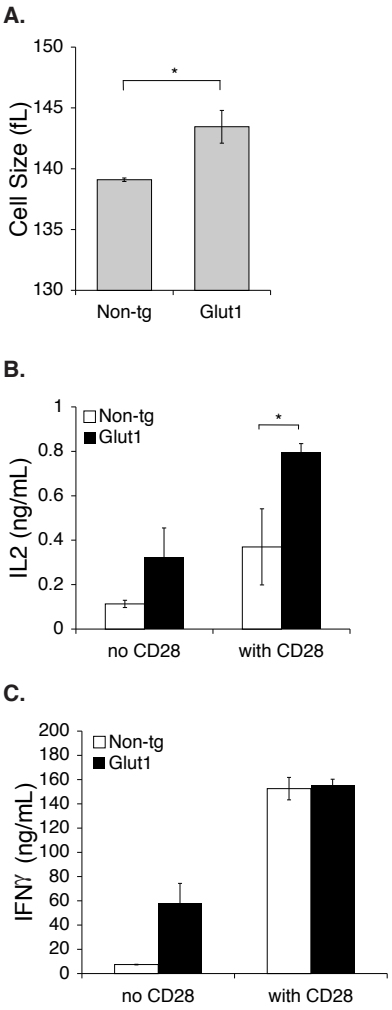

Figure 3.6: Transgenic Glut1 over-expression increases T cell size and cytokine production.

A. The size of resting T cells from Glut1 transgenic and non-transgenic animals was determined by particle size analyzer (* indicates $p < 0.005$). **B.** T cells were stimulated 0.5μg/mL anti-CD3, with or without 5μg/mL anti-CD28, for 2 days and supernatant was collected and analyzed by ELISA for IL2 production (* indicates $p < 0.05$). **C.** T cells were stimulated with 5μg/mL anti-CD3, with or without 5μg/mL anti-CD28, for 3 days and supernatant was collected and analyzed by ELISA for IFNγ production.

developing or resting T cells *in vivo* as neither T cell development nor homeostasis were grossly altered in young Glut1 transgenic mice.

3.2.6 Overexpression of Glut1 alters cell size and cytokine production

Although many T cell phenotypes were not altered by Glut1 transgene expression and increased glucose uptake capacity, some key differences between Glut1 and non-transgenic T cells were observed. First, naive purified transgenic cells were significantly larger in size than non-transgenic cells as determined with a particle size analyzer ($p < 0.005$); (Figure 3.6A). This is consistent with previous observations that neglected T cells have reduced nutrient uptake and cell size, while T cells that expressed activated Akt have elevated glucose metabolism and increased cell size (Rathmell et al., 2003a; Rathmell et al., 2000). Second, Glut1 transgenic cells also showed an increased ability to produce IL2 and IFNγ during T cell activation. Purified T cells were stimulated with anti-TCR alone or with anti-CD28, and cytokine production was analyzed after two days. Non-transgenic T cells were highly dependent on CD28 for maximal IL2 production, while Glut1 transgenic T cells produced more IL2 and were less dependent on CD28 to promote IL2 secretion (Figure 3.6B). Similarly, over-expression of Glut1 allowed for increased CD28-independent production of IFNγ (Figure 3.6C). However, the increased IFNγ production by Glut1 transgenic T cells was not evident when cells were costimulated with CD28, as non-transgenic T cells matched IFNγ production of Glut1 transgenic T cells. These data show that increased expression of Glut1 is sufficient to augment both T cell growth and cytokine secretion upon stimulation, demonstrating that glucose uptake is a limiting factor for T cell growth and stimulation.

3.2.7 Chronic exposure to Glut1 over-expression results in increased T cell activation *in vivo*

Despite the generally normal phenotype of young Glut1-transgenic mice, the enhanced *in vitro* activation of Glut1-transgenic T cells suggested that T cells may accumulate a previously-activated or memory phenotype *in vivo* with age. To investigate

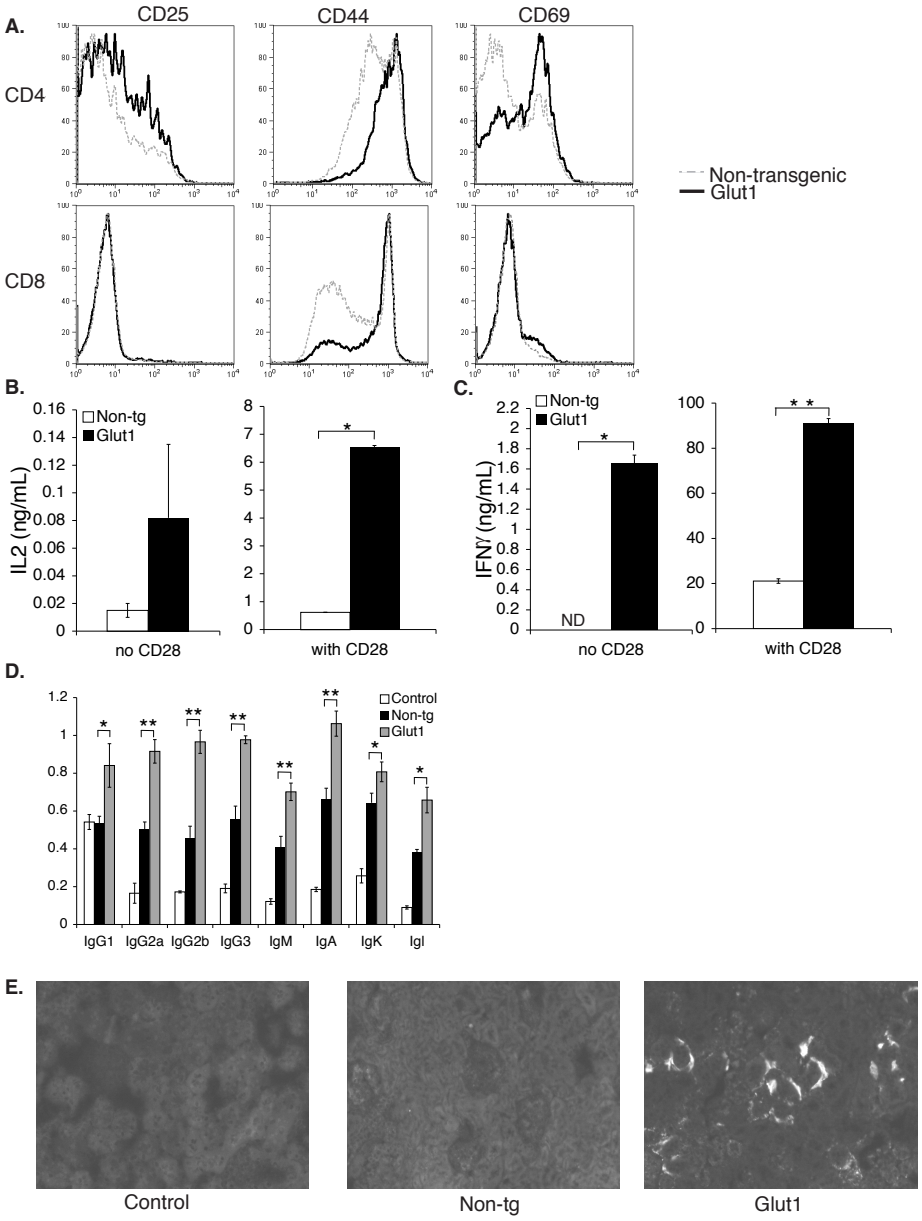

Figure 3.7: Glut1 over-expression led to accumulation of memory-phenotype T cells in aged mice.

A. Resting T cells purified from Glut1 transgenic and age-matched non-transgenic animals over 1 year old were stained with fluorescently labeled antibodies to detect the T cell activation markers CD25, CD44, and CD69 and analyzed by flow cytometry. **B, C.** Resting T cells purified from Glut1 transgenic and age-matched non-transgenic animals over 1 year old were stimulated with 1μg/mL anti-CD3, with or without 5μg/mL anti-CD28, for 1 day and supernatants were collected and analyzed for cytokine production by ELISA (*$p < 0.05$, ** $p < 0.005$, ND = Not Detected). **D.** Serum from representative aged matched Glut1 transgenic and non-transgenic mice was analyzed by ELISA for immunoglobulin isotypes (*$p < 0.04$, ** $p < 0.01$). **E.** Kidney sections from eight week old and year old aged matched Glut1 transgenic and non-transgenic mice were stained for anti-Mouse immunoglobulin and examined microscopically.

this possibility, one-year-old Glut1 and non-transgenic littermates were analyzed. Transgenic mice showed no overt signs of illness, yet flow cytometric analysis demonstrated an increased percentage of CD4+ T cells with elevated levels of the activation markers CD25, CD44, and CD69 in old Glut1 transgenic mice compared to age-matched non-transgenic mice (Figure 3.7A). An increase in CD44 staining was also observed in Glut1 transgenic CD8+ T cells. Consistent with a previously-activated or memory phenotype *in vivo*, i*n vitro* stimulation of aged Glut1 T cells resulted in greatly increased production of both IL2 and IFNγ (Figure 3.7B and C; ND, not detectable). T cells from Glut1 transgenic mice produced more of both cytokines than non-transgenic cells following a one-day stimulation with CD3 alone, as well as with CD28 costimulation.

To further determine the impact of Glut1 over-expression on immune homeostasis, levels of immunoglobulin isotypes were determined in the serum of one-year-old Glut1 transgenic mice and non-transgenic littermates. All immunoglobulin isotypes were higher in the Glut1 transgenic mice than non-transgenic littermates (Figure 3.7D). In addition, Glut1 transgenic mice had increased immunoglobulin deposits in the kidneys (Figure 3.7E). Together, these data demonstrate that glucose uptake is a limiting aspect of T cell activation and that increased Glut1 expression is capable of promoting accumulation of memory-phenotype T cells that can promote B cell antibody production and possible immune pathology in aged animals.

3.2.8 Akt signaling plays a role in the regulation of glucose uptake but cannot fully substitute for CD28 signaling effects on glucose uptake

These data show that the increase in glucose uptake that occurs upon CD28-mediated costimulation is vital for a proper immune response, and chronically increased uptake alone is capable of promoting T cell activation, particularly in aged mice. However, it remained unclear how glucose uptake was upregulated by CD28 costimulation. Activation of the PI3K/Akt pathway has been implicated as a transducer of CD28 signals to increase glucose uptake following T cell stimulation (Frauwirth et al., 2002). Therefore, we sought to determine the role of the Akt pathway in CD28-dependent upregulation of Glut1 expression and glucose uptake. T cells were purified from Akt1-/- animals and stimulated with a low level of anti-CD3 with or without CD28 costimulation. Despite the continued presence of Akt2 and Akt3, which are expressed in T cells and can also regulate glucose uptake (Juntilla et al., 2007), glucose uptake appeared to be reduced in Akt1-/- costimulated T cells compared to Akt1+/+ T cells (Figure 3.8A) suggesting a role for this Akt isoform in T cell glucose uptake. To determine the sufficiency of Akt signaling in CD28-mediated regulation of glucose uptake, T cells were isolated from transgenic mice that express constitutively active myristoylated Akt1 (mAkt) in T cells and from non-transgenic littermates. Expression of the mAkt transgene has been shown to decrease T cell dependence on CD28 for cell growth, cytokine production, and proliferation (Rathmell et al., 2003a). Akt activation due to transgenic expression was comparable or higher than that achieved in non-transgenic cells by CD3 stimulation and CD28-costimulation as determined by phospho-Akt levels one hour after stimulation (Figure 3.8B). Therefore, transgenic mAkt expression provided a model to analyze the role of this pathway in CD28 signaling to stimulate glucose uptake.

Akt may promote glucose uptake through multiple mechanisms that include both regulation of protein expression as well as post-translational control of Glut1

protein trafficking and activity (Barthel et al., 1999; Edinger and Thompson, 2002; Plas et al., 2001; Wieman et al., 2007). Therefore Glut1 protein levels were observed in resting and stimulated T cells from non- or mAkt-transgenic mice. Expression of mAkt was found to have no discernible effect on total Glut1 protein levels in unstimulated, stimulated, or costimulated T cells after one day (Figure 3.8C). Although mAkt did not appear to affect Glut1 protein levels, Glut1 glycosylation was regulated by PI3K activity, as treatment of cells with the PI3K inhibitor LY294002 (LY) resulted in a decrease in the mobility shift normally seen in co-stimulated T cells at both the low and high dose of anti-CD3 (Figure 3.8D). Despite the apparent inability of Akt activity to alter Glut1 protein levels, glucose uptake of unstimulated mAkt T cells was significantly higher than that of unstimulated non-transgenic T cells ($p< 0.02$) (Figure 3.8E). These data suggest that Akt may not regulate Glut1 expression, but consistent with the role of Akt to regulate Glut1 trafficking in response to cytokine signaling (Bentley et al., 2003; Wieman et al., 2007; Wofford et al., 2008), may increase Glut1 surface trafficking or glucose uptake activity.

The ability of Akt to stimulate Glut1 surface trafficking and activity may comprise a significant portion or all CD28-mediated glucose uptake. To determine if Akt activation was sufficient to replace CD28-stimulated glucose uptake in T cell activation, non-transgenic and mAkt transgenic T cells were stimulated for one day and glucose uptake was analyzed (Figure 3.8F). In the absence of CD28 costimulation, a low dose of anti-CD3, which was incapable of inducing Glut1 protein (Figure 3.2B), modestly increased glucose uptake in non-transgenic T cells. Glucose uptake of CD28-costimulated T cells increased compared to CD3-only stimulated cells. Importantly, mAkt expression was not capable of mimicking CD28 costimulation and failed to induce additional glucose uptake in T cells stimulated with a low dose of anti-CD3 alone. In

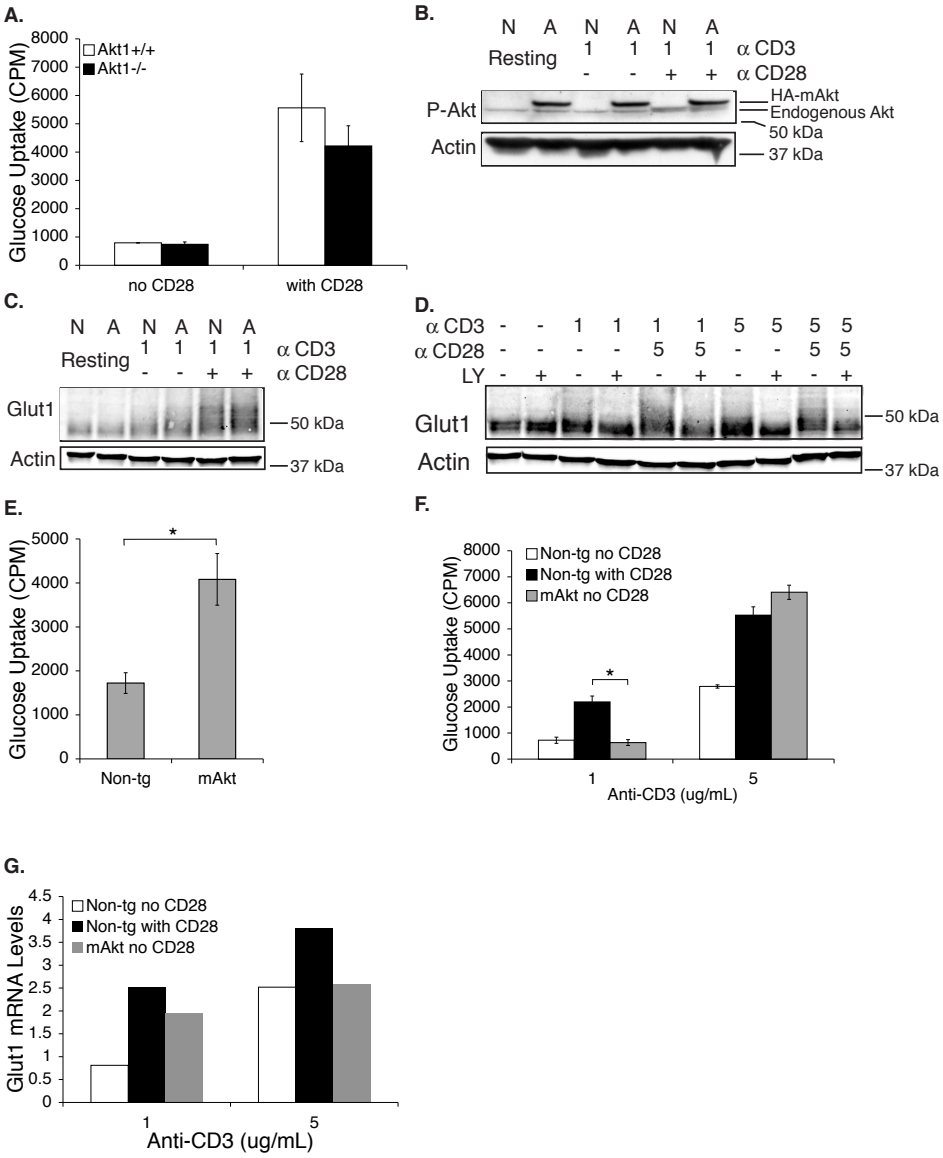

Figure 3.8: Constitutively active Akt increases glucose uptake in resting cells but cannot compensate for CD28 signals at low levels of CD3 stimulation.

A. T cells from Akt1-/- animals and Akt1+/+ littermates were purified, stimulated with 1μg/mL anti-CD3 with and without 5μg/mL anti-CD28 for 18 hours and glucose uptake and Glut1 protein levels were analyzed. **B, C.** T cells were purified from mAkt transgenic (A) and non-transgenic (N) littermates and stimulated with CD3 with and without 5μg/mL anti-CD28 **(B)** for one hour prior to cell lysis and analysis by immunoblotting for phospho-Akt which appeared larger in the transgenic mice than the non-transgenic due to the presence of an HA tag in the transgene and **(C)** for 1 day prior to cell lysis and analysis by immunoblotting for Glut1. **D.** Purified T cells were pretreated with LY294002 or vehicle control for 30 minutes and stimulated with anti-CD3 and anti-CD28 at the indicated concentrations for 1 day prior to T cell lysis and immunoblotting for Glut1. **E.** Purified resting T cells from mAkt and non-transgenic animals were analyzed for glucose uptake (* indicates $p < 0.02$). **F, G.** T cells purified from mAkt and non-transgenic animals were stimulated with the indicated dose of anti-CD3 with and without 5μg/mL anti-CD28 for 1 day prior to (**F**) analysis of glucose uptake (* indicates $p < 0.005$) and (**G)** RNA extraction and qRT-PCR analysis of Glut1 mRNA levels.

contrast, at higher doses of anti-CD3 where Glut1 protein was induced in the absence of CD28 costimulation (Figure 3.2B), Akt activation was sufficient to augment glucose uptake to an extent similar to that seen in non-transgenic costimulated cells. Therefore, while Akt signals may play an important role in CD28-mediated glucose uptake, Akt is not sufficient to replace CD28-mediated increases in glucose uptake in weakly TCR stimulated cells. Although constitutively active Akt was not capable of substituting for CD28 costimulation effects on glucose uptake, mAkt expression increased glut1 message following stimulation with a low dose of anti-CD3 to levels similar to costimulated non-transgenic T cells. However, a high does of anti-CD3 stimulation in mAkt expressing T cells did not induce glut1 message levels to those seen during costimulation of control cells (Figure 3.8G). This suggested that mAkt was able to increase glucose uptake and augment glut1 message levels, although this increase in message did not result in an increase in Glut1 protein or glucose uptake. Together, these data suggest that pathways other than Akt mediated by TCR or CD28-stimulation, such as those that lead to Glut1 induction or modification, are required for Akt to promote glucose uptake.

3.2.9 Glut1 and Akt additively increase T cell stimulation

As Akt did not lead to induction of Glut1 protein in CD3-stimulated cells, we sought to determine if directly increased expression of Glut1 may alter the role of Akt in T cell costimulation. For this purpose, Glut1/mAkt double transgenic animals were generated and T cells from double transgenic mice were compared to Glut1 and mAkt single transgenic and non-transgenic littermates. Importantly, double transgenic cells demonstrated a synergistic increase in glucose uptake compared to either single transgenic (Figure 3.9A). Resting T cells purified from young non-transgenic, Glut1, mAkt, and Glut1/mAkt transgenic mice also had genotype-dependent variations in cell

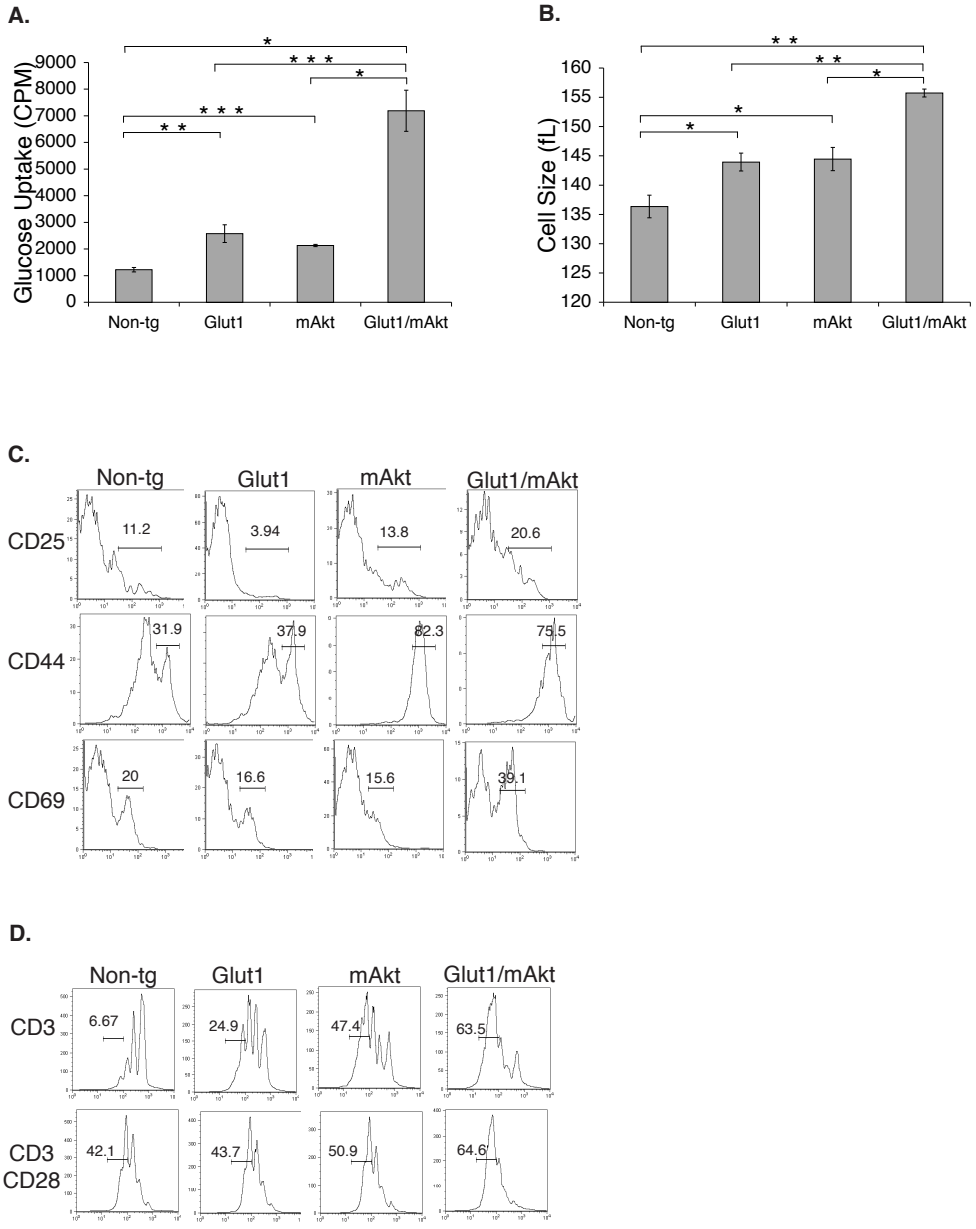

Figure 3.9: T cell glucose uptake and activation are increased in Glut1 and mAkt double transgenic animals.

A. Glucose uptake was analyzed on purified resting T cells from littermates (* indicates $p < 0.01$, ** $p < 0.05$, *** $p < 0.005$). **B.** Cell size was determined by particle size analyzer analysis on purified resting T cells from non-transgenic, Glut1, mAkt, and Glut1/mAkt double transgenic cells (* $p < 0.01$ and ** $p < 0.005$). **C.** Levels of T cell activation markers CD25, CD44, and CD69 were determined with fluorescently labeled antibodies and flow cytometric analyses of resting purified T cells from the indicated genotypes. **D.** Purified T cells were stained with CFSE, stimulated with 5μg/mL anti-CD3, with and without 5μg/mL anti-CD28, for three days, and levels of CFSE depletion to indicate cell proliferation were determined by flow cytometry.

size as determined by a particle size analyzer (Figure 3.9B). Glut1 and mAkt transgenic cells were similar in size to each other yet larger than non-transgenic cells. The increased T cell size and glucose uptake observed in the presence of Akt activation or Glut1 over-expression suggested that these two pathways may synergize to promote cell growth. Consistent with this notion, double transgenic T cells were significantly larger than cells from either single transgenic ($p < 0.005$ and $p < 0.01$). In addition, T cells in Glut1/mAkt double transgenic mice had a more memory-like phenotype than either single transgenic. As shown above (Figure 3.5E), expression of the Glut1 transgene alone did not alter expression of CD25 or CD44 on resting CD4 T cells. As previously reported, CD4 T cells expressing the mAkt transgene had increased expression of CD44 (Figure 3.9C) (Rathmell et al., 2003a). However, double transgenic CD4 T cells had higher expression of CD25, CD44, and CD69 activation markers than either single transgenic. These data indicate that increases in Glut1 protein levels and Akt signaling combined to further augment glucose uptake, T cell activation, and accumulation of previously-activated T cells.

In addition to increased activation of T cells *in vivo*, transgenic expression of Glut1 and mAkt enhanced T cell activation after *in vitro* stimulation and further reduced T cell dependence on CD28 costimulation. To examine T cell activation, T cells from non-, mAkt, Glut1 and mAkt/Glut1 transgenic mice were labeled with CFSE and stimulated with anti-CD3 with or without anti-CD28 and were evaluated after three days to determine proliferation levels. When stimulated by anti-CD3 alone, Glut1 transgenic cells proliferated more than non-transgenic cells, mAkt transgenic cells more than Glut1 cells, and double transgenic cells more than either single transgenic (Figure 3.9D). A similar trend was observed with anti-CD3 and CD28 costimulation but with smaller differences due to increased proliferation of non-transgenic cells. Comparable levels of

proliferation of Glut1/mAkt cells were observed in anti-CD3 and anti-CD3 plus CD28 costimulation cultures, indicating that double transgenic expression results in CD28-independence. These data demonstrate that upregulation of Glut1 is a critical component of T cell activation and, in combination with Akt activation, which may promote Glut1 surface trafficking and activity, can largely replace the CD28 T cell costimulatory signal.

3.3 Discussion

In this study we addressed the role of glucose uptake in T cell development, homeostasis, and activation as well as explored the mechanism by which the CD28 costimulatory signal regulates glucose uptake. Limiting glucose uptake resulted in decreased T cell proliferation and IFNγ production, while increasing Glut1 protein levels through transgenic expression did not appear to affect T cell development or homeostasis, but did result in increased cell size and cytokine production upon activation. In addition, aged Glut1 transgenic mice showed increased immunoglobulin levels in the serum and deposition in the kidney. We implicate the involvement of the PI3K/Akt pathway (Frauwirth et al., 2002; Rathmell et al., 2003a), and demonstrate that other CD28-signaling pathways are also required to augment TCR-mediated induction of Glut1. In particular, constitutive activation of Akt was sufficient to increase glucose uptake in resting T cells, but was insufficient to achieve levels of glucose uptake seen during costimulation unless Glut1 protein was upregulated independently by strong TCR signals. This suggested that Akt activation downstream of CD28 signaling may regulate Glut1 surface localization or activity, but a separate pathway was necessary to upregulate Glut1 protein (Figure 3.10). Together, these data indicate that regulation of glucose uptake is a critical aspect of T cell activation, and that Akt-independent

regulation of Glut1 expression and Akt-dependent regulation of Glut1 trafficking and glucose uptake activity may account for the central role of CD28 in glucose metabolism of activated T cells.

This work delineates a vital role for glucose and glucose uptake in immunity. Despite availability of other nutrients, glucose was required for increased growth, IL2 production, and proliferation upon T cell stimulation. Conversely, increased T cell activation that occurred when Glut1 was over-expressed suggested that glucose uptake is normally limiting in T cell stimulation. It should be noted, however, that in addition to decreased glucose metabolism, glucose limitation can lead to ER stress and activation of

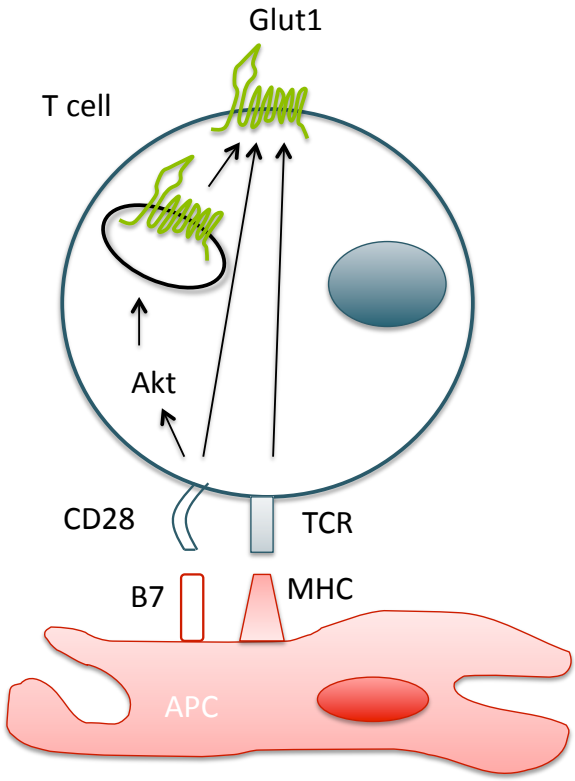

Figure 3.10: Model of TCR and costimulation effects on Glut1

TCR signaling increases Glut1 protein levels and this can be augmented by CD28 costimulation. CD28 signaling also activates Akt, which promotes localization of Glut1 to the cell surface.

AMPK that may complicate these analyses (Jones et al., 2005). Nevertheless, Glut1 transgenic T cells demonstrate that this level of increased glucose uptake is insufficient to allow maximal immune response. The extensive increase (>10 fold) in glucose uptake that occurs after one day of stimulation (Figure 3.1A) relative to the increase in glucose uptake provided by the Glut1 transgene (3-4 fold; Figure 3.5B) suggests that glucose may be particularly limiting in the early phases of T cell activation. Consistent with glucose limitation early in T cell activation, Tamás et al (2006) recently showed an important role for AMPK activation following TCR stimulation, which may promote more efficient ATP generation or use of alternative fuels (Tamas et al., 2006) while Glut1 protein is synthesized and glucose uptake increases to meet cellular demands. While increases in energy availability are necessary for early T cell activation, maintaining the appropriate balance of nutrient uptake and energy production is also critical for T cell homeostasis as increased glucose uptake led to possible immune pathology with age.

Blockade of costimulation with CTLA4-Ig or anti-ICAM on CD3-stimulated T cells cultured on LPS-stimulated macrophages prevented the maximal increase of glucose uptake in T cells. Despite the wide variety of other cell-cell interactions and soluble factors that LPS-activated macrophages may provide, these costimulation pathways provide a necessary combination of signals for the regulation of T cell glucose uptake upon activation. Common features of co-stimulation include enhancement of TCR signals and activation of PI3K/Akt. The combination of these signaling pathways may be critical for upregulation of glucose uptake. Conversely, receptors that decrease these signaling pathways, such as CTLA4, may decrease glucose uptake. Although signals downstream of CD28 that regulate glucose metabolism are not entirely clear, the PI3K/Akt pathway appears to play a critical role. The Akt pathway is well known to control trafficking of the insulin sensitive glucose transporter Glut4 (Dugani and Klip,

2005), and we have directly shown Akt regulation of Glut1 cell surface trafficking in a lymphoid cell line in response to cytokine signaling (Wieman et al., 2007; Wofford et al., 2008). Akt has also been shown to regulate cell surface trafficking of Glut1 in the mammary gland and was required to meet the increased metabolic demand associated with lactation (Boxer et al., 2006). In addition to regulation of cell surface levels, Akt may also control Glut1 transporter activity through activation of the mammalian target of rapamycin (mTOR) (Wieman et al., 2007). Glut1 glycosylation also appeared to be PI3K-dependent, although Akt activation was not sufficient to substantially alter this Glut1 modification. Therefore, Akt may play a critical role in response to costimulation to promote trafficking of Glut1 protein to the cell surface and to stimulate Glut1 activity.

The capacity of Akt signaling to control Glut1 localization or activity may combine with pathways that upregulate or modify Glut1 to jointly mediate CD28-induced glucose uptake. In addition to activation of the PI3K/Akt pathway, CD28 also augments CD3 signals that may play a critical and underappreciated role to upregulate and modify Glut1 protein. Other likely signaling pathways that may regulate Glut1 protein levels downstream of CD28 include activation of the TEC kinases, TEC and ITK, which can play a role in positive regulation of PLC- γ (Michel et al., 2001). Another signaling pathway that may link CD28 to increased Glut1 protein levels is the small GTPase GEF, Vav1 (Turner and Billadeau, 2002). Vav1 associates with the membrane in a PI3K-independent mechanism (Michel et al., 1998) and is known to potently induce activation of NFAT, NF-κB, and AP1 (Michel et al., 2000). Each of these transcription factors could be responsible for induction of Glut1 protein expression downstream of CD28 and explain how CD3 signals alone, when strong enough, are capable of inducing increased Glut1 expression.

Increased glucose uptake may benefit T cell activation through a number of mechanisms. T cell stimulation increases the intracellular need for ATP and other high energy molecules (Buttgereit et al., 2000). This need is met primarily by an increase in glycolysis, resulting in substantial lactate production (Bental and Deutsch, 1993). However, glycolysis is not the only cellular metabolic pathway that may benefit from increased intracellular glucose levels. The pentose phosphate pathway, which produces pentose sugars required for nucleic acid synthesis and NADPH, for reducing power and lipid synthesis, is also upregulated in response to T cell activation (Sagone et al., 1974). In addition, increased glucose metabolism may alter signaling pathways to enhance activation and survival. In both hematopoietic cell lines and Glut1 transgenic T cells, we have shown elevated levels of glucose metabolism to lead to increased levels of phospho-GSK3αβ (Zhao et al., 2007). This inhibitory phosphorylation of GSK3 may augment cellular activation by reducing nuclear export of transcription factors such as NFAT (Macian, 2005) as well as protect against apoptosis by stabilizing the anti-apoptotic Bcl-2 family member Mcl-1 (Maurer et al., 2006; Zhao et al., 2007). Together, these pathways establish signaling pathway coordination between nutrient sensing, proliferation, and cell survival.

The evidence presented here implicates regulation of glucose uptake and Glut1 as critical features of immune function. In diabetes research, it has become clear that pathways that regulate glucose transporter translocation are a central aspect of insulin-dependent promotion of metabolism and growth of insulin-responsive tissues. T cells do not typically respond directly to insulin, yet comparably to insulin regulation of Glut4, CD28 provides a signal to T cell metabolism and growth that is mediated by induction of Glut1 and regulation of Glut1 trafficking. Similar to T cell activation, cancer cells also often show elevated Glut1 expression and glycolytic metabolism (Semenza et al., 2001;

Warburg, 1956). Understanding the regulatory pathways underlying this metabolic phenotype may therefore provide useful insight into immunological and neoplastic diseases, as well as illustrate the critical role that nutrition may have on immune function.

4. Interleukin-7 is essential to maintain T cell glycolysis *in vivo*

4.1 Introduction

IL-7 has emerged as a unique cytokine required for the development and homeostasis of the adaptive immune system. Originally identified as a growth promoting factor for B cells (Namen et al., 1988), IL-7 has since been described as a factor necessary for the development of B cells and T cells in mice, development of T cells in humans, homeostatic T cell proliferation, and survival of naive T cells. IL-7, produced by stromal cells, is detected by a two-part receptor on lymphocytes consisting of the common gamma chain (γ_c) shared by multiple cytokines and a more specific receptor, denoted IL-7Rα (IL-7R).

IL-7 may maintain naive T cell survival through the regulation of Bcl-2 family members. Specifically, IL-7 signaling results in an increased expression in the anti-apoptotic protein Bcl-2 (Kim et al., 1998), and over expression of Bcl-2 can partially rescue T cell development in IL-7R knockout animals (IL-7R$^{-/-}$) (Akashi et al., 1997; Maraskovsky et al., 1997). A second anti-apoptotic Bcl-2 family member, Mcl-1, has also been connected to IL-7 induced cell survival, as conditional knockout of Mcl-1 results in reduced numbers of B and T cells, and IL-7 dependent cell survival was eliminated in the absence of Mcl-1 (Opferman et al., 2003). Pro-apoptotic Bcl-2 family members Bim and Bax have also been implicated in the ability of IL-7 to maintain survival (Khaled et al., 2002; Pellegrini et al., 2004). Although genetic models to modify expression of Bcl-2 family members can partially rescue loss of IL-7 signaling, no single modification in these apoptotic genes has completely restored the defects associated with the loss of IL-7. For example, Bcl-2 over expression in an IL-7R$^{-/-}$ background resulted in partial

rescue of T cell development and numbers but lacked the ability to produce γδ T cells (Akashi et al., 1997), and IL-7 can still inhibit death in Bcl-2 deficient T cells (Nakayama et al., 1995).

Pathways downstream of the IL-7R other than regulation of Bcl-2 family members may be responsible for the inability of Bcl-2 to completely rescue T cell development in IL-7R$^{-/-}$ animals. These pathways include signaling through the transcription factor STAT5 to activate Pim kinases and Akt (Plas et al., 2002; Wofford et al., 2008). Both Pim kinases (Fox et al., 2003) and Akt have been shown to regulate glucose metabolism in a variety of systems, including lymphoid cells (Doughty et al., 2006; Rathmell et al., 2003a; Wieman et al., 2009), which led to the hypothesis that IL-7 may regulate cell survival and function at least in part by promoting glucose metabolism. Data from *in vitro* experimentation supports a role for IL-7 in the regulation of glucose metabolism. T cells isolated and cultured in the absence of normal environmental signals such as IL-7 have decreased glucose uptake and glycolysis. Culture of T cells in the presence of IL-7, however, can partially maintain glucose uptake and can wholly maintain T cell glycolytic flux (Rathmell et al., 2001; Wofford et al., 2008). Although regulation of glucose metabolism by addition of recombinant IL-7 has been demonstrated *in vitro*, no evidence has emerged to indicate that this regulation has a functional role *in vivo* with endogenous levels of IL-7.

Work described in this chapter addresses the *in vivo* role of IL-7 in the regulation of glucose metabolism. Initial experiments were performed by adoptively transferring wildtype T cells into IL-7$^{-/-}$ hosts. However, this technique allowed only limited biochemical analysis of transferred T cells due to the low number of cells that could be recovered by cell sorting. An inducible IL-7R knockout model was therefore produced. This novel transgenic mouse, which expressed the IL-7R under a T cell specific promoter

flanked by *loxP* sites, was used to allow conditional rescue of expression of IL-7R in IL-7R$^{-/-}$ animals. Transgenic expression of IL-7R supported T cell development and produced mature T cells that responded normally to IL-7. Upon injection of Tamoxifen to activate the Cre:ER recombinase, the IL-7R transgene was excised. This resulted in a decrease in T cell size, number, survival, and a lack of response to IL-7 signaling. Loss of IL-7 signaling *in vivo* resulted in no apparent change in levels of Bcl-2 family members and, surprisingly, an increase in the total amount of Glut1 protein, the primary glucose transporter in lymphocytes (Chakrabarti et al., 1994). However, T cells rendered IL-7R deficient *in vivo* atrophied and failed to maintain normal glycolytic flux. This study, therefore, identifies a novel role for IL-7 in regulating glucose metabolism through maintenance of glycolysis *in vivo*.

4.2 Results

4.2.1 IL-7 regulates cell survival and cell death *in vivo*

Previous reports indicate that IL-7 can regulate glucose metabolism of primary lymphocytes *in vitro* (Rathmell et al., 2001; Wofford et al., 2008). Validation for this role of IL-7 *in vivo* has been difficult to address, as IL-7 is required for T cell development (von Freeden-Jeffry et al., 1995) and normally selected mature T cells are not present in IL-7R$^{-/-}$ or IL-7$^{-/-}$ animals (Peschon et al., 1994). To determine if IL-7 signaling regulates glucose metabolism in naive T cells *in vivo*, primary T cells purified from wildtype animals were adoptively transferred into IL-7$^{-/-}$ hosts. Consistent with previous results (Schluns et al., 2000; Tan et al., 2001), the number of transferred cells was reduced in the absence of IL-7 compared to heterogeneous controls (Figure 4.1A), suggesting that the lack of cytokine resulted in an increase in cell death *in vivo*. Additionally, loss of IL-7 resulted in T cell atrophy, as mean forward scatter was reduced in both CD4+ and CD8+

T cells transferred into IL-7$^{-/-}$ hosts compared to control (Figure 4.1B). This decrease in cell size *in vivo* mimicked that seen following growth factor withdrawal *in vitro*. The relationship between cell size and glucose metabolism from *in vitro* work suggested that this decrease in cell size may have been due to a decrease in available energy. After one week, adoptively transferred wildtype T cells were sorted from IL-7$^{-/-}$ host spleens and analyzed for levels of glut1 mRNA by real time quantitative rtPCR. Message levels of glut1 were not significantly different between IL-7$^{-/-}$ and IL-7$^{+/-}$ hosts (Figure 4.1C). Additionally, no functional difference in the ability to uptake glucose was observed as measured in T cells 7 days post-transfer (Figure 4.1D). Due to the small number of

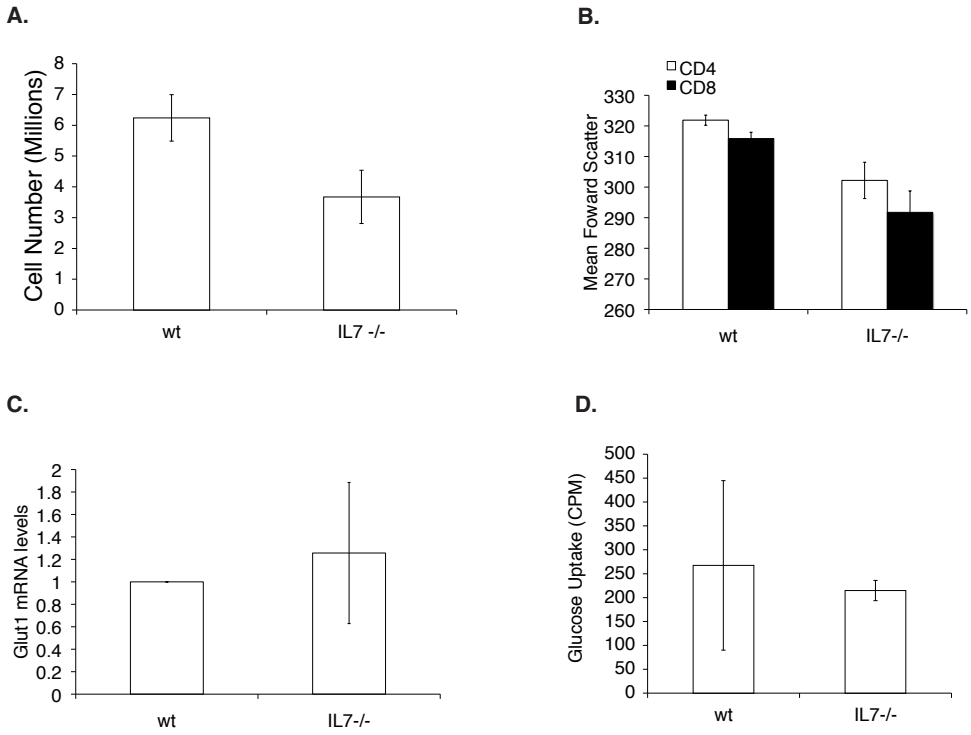

Figure 4.1: IL-7 effects cell survival in adoptive transfer experiments.

Wildtype T cells were stained with CFSE and adoptively transferred into IL-7$^{-/-}$ animals for seven days. **A.** The number of transferred cells remaining in the spleen was determined by flow cytometry and counting the number of CFSE positive cells remaining in IL-7$^{-/-}$ or wt hosts. **B.** Cell size of adoptively transferred cells was determined by flow cytometry and mean forward scatter. **C.** Transferred cells were sorted from host spleens and subjected to real time qRT-PCR analysis of Glut1 protein levels. **D.** Transferred cells were sorted from host spleens and glucose uptake analysis was preformed.

adoptively transferred T cells recovered by cell sorting of host spleens, these metabolic assays were performed with the minimum cell number technically allowed. It was unclear, therefore, if these results were indicative of the role of IL-7, or a lack of signal due to technical challenges. To address IL-7 regulation of glucose metabolism *in vivo* more completely, an inducible IL-7R Cre/Lox transgenic system was produced.

4.2.2 Generation of an inducible IL-7R knockout system

To allow for biochemical analysis and determination of the role of IL-7 *in vivo*, we generated a novel inducible knockout of the IL-7R. As the IL-7R is expressed specifically on cells derived from the hematopoietic system, it was possible to replace the endogenous IL-7R with transgenic expression via a T cell specific promoter. This allowed for rapid generation of a transgenic IL-7R flanked by *loxP* sites as opposed to the technically challenging process of generating a floxed knock-in IL-7R. Not only was this approach faster than a conventional knock-in model, but it allowed for tissue specific expression of our target gene, and transgenic animals that expressed the floxed IL-7R via the T cell specific Lck promoter (floxed IL-7R tg) were used to rescue IL-7R expression in IL-7R$^{-/-}$ animals that were commercially available (Figure 4.2). This produced animals with a single locus of the IL-7R flanked by *loxP* sites, which could be excised by expression or activation of Cre recombinase. The restricted expression of the IL-7R to the hematopoietic system allowed for the use of a non-tissue specific, drug inducible Cre recombinase transgene. Transgenic animals that expressed Cre recombinase fused to a portion of the estrogen receptor (Cre:ER), which is inducible only by the selective estrogen receptor modulator, Tamoxifen, were bred onto the IL-7R$^{-/-}$ background. This transgene was expressed under a ubiquitin promoter, allowing the recombinase to translocate into the nucleus in all cells, but resulting in loss of the IL-7R only in T cells where the floxed IL-7R transgene is expressed. Animals containing the

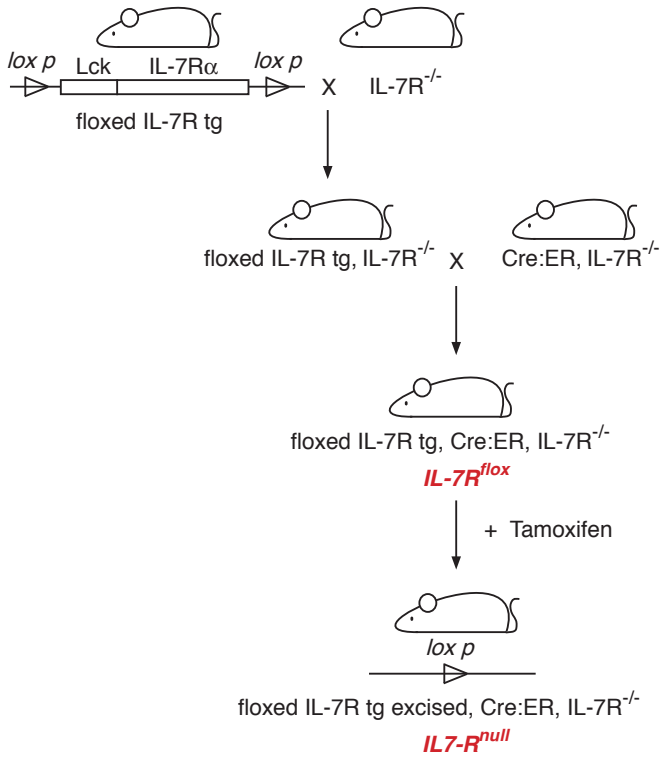

Figure 4.2: Model of inducible IL-7R knockout.

Diagram of the generation of the IL-7R^{flox} transgene, rescue of IL-7R$^{-/-}$ with the transgene, and incorporation of a Tamoxifen inducible Cre to allow for *in vivo* excision of the transgene.

floxed IL-7R transgene on a IL-7R$^{-/-}$ background are denoted IL-7R^{flox}. When treated with Tamoxifen by intraperitoneal injection, the IL-7R transgene can be excised *in vivo* to produce IL-7R^{null} animals. This transgenic rescue of the knockout system was ideal to study the role of IL-7 in mature T cells and allowed for tissue specific, fast, simple creation of an inducible system and avoided of the challenges associated with the generation of a knock-in mouse.

4.2.3 IL-7R^{flox} transgene largely rescues IL-7R$^{-/-}$ phenotype

We first sought to determine the effectiveness of the IL-7R^{flox} transgene to produce functional, mature T cells on the IL-7R$^{-/-}$ background by comparing IL-7R^{flox} and

IL-7R$^{-/-}$ to wildtype (wt) controls. As expected, the number of cells present in the thymus and spleen of IL-7R$^{-/-}$ was significantly reduced compared to wildtype, and expression of the IL-7R^{flox} transgene resulted in an effective restoration of cell numbers in these tissues compared to IL-7R$^{-/-}$ (Figure 4.3A). The difference in cell number in the spleen and thymus of IL-7R^{flox} compared to wildtype animals may have been due in part to a reduction of IL-7R levels in the IL-7R^{flox} animals in both thymus and mature splenic T cells, although IL-7R levels in IL-7R^{flox} animals were significantly higher than those of IL-7R$^{-/-}$ animals (Figure 4.3B). No significant differences were detectable in the cell size of thymocytes or splenocytes between wt, IL-7R$^{-/-}$, and IL-7R^{flox} animals (Figure 4.3C). These data indicated that the floxed IL-7R transgene was capable of being transcribed, translated, and localized to the cell membrane to effectively rescue T cell numbers.

Recovery of cell surface IL-7R levels and thymic and splenic cell numbers by expression of the IL-7R^{flox} transgene on an IL-7R$^{-/-}$ background suggested that the transgene could functionally replace IL-7R signaling for T cell development. Consistent with this, CD4+ to CD8+ ratios in IL-7R^{flox} animals mimicked those of wildtype in the thymus and spleen (Figure 4.3D). We also examined the functionality of the IL-7R^{flox} transgene in cells by determining the ability of IL-7 to maintain cell survival *in vitro*. Endogenous and IL-7R^{flox} rescued T cells cultured in the presence of IL-7 maintained an approximately 80% survival rate, while without IL-7 only 10% survival was achieved in both wildtype and IL-7R^{flox} cells after three days (Figure 4.3E). This demonstrated that the transgenic receptor was able to use IL-7 as effectively as endogenous T cells to maintain cell survival *in vitro*. As IL-7 has been implicated in maintaining cell survival through the regulation and balance of Bcl-2 family members (Khaled et al., 2002; Kim et al., 1998), the levels of Bcl-2 and Mcl-1 in wildtype and IL-7R^{flox} rescued T cells *ex vivo*

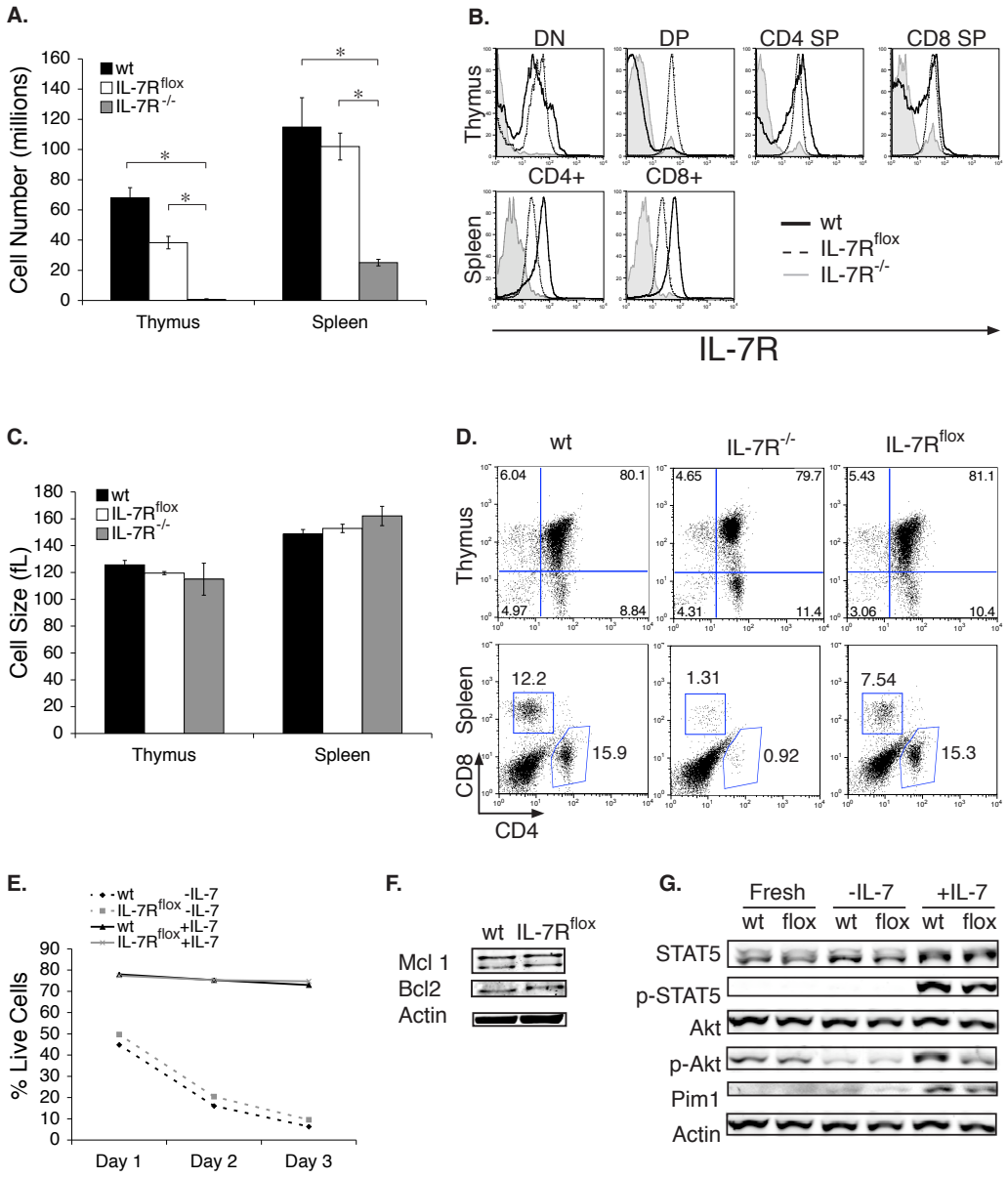

Figure 4.3: IL-7R^{flox} transgene rescues T cell development in IL-7R$^{-/-}$ animals.

A. Cell number of thymocytes and splenocytes from wildtype, IL-7R^{flox}, and IL-7R$^{-/-}$ animals were determined by particle size analyzer (*, $p < 0.05$). **B.** Thymocytes and splenocytes from wildtype, IL-7R^{flox}, and IL-7R$^{-/-}$ animals were fluorescently labeled with antibodies against CD4, CD8 and IL-7R and analyzed by flow cytometry to determine IL-7R levels. **C.** Cell size of thymocytes and splenocytes from wildtype, IL-7R^{flox}, and IL-7R$^{-/-}$ animals were determined by particle size analyzer **D.** Thymocytes and splenocytes from wildtype, IL-7R^{flox}, and IL-7R$^{-/-}$ animals were stained with fluorescently conjugated antibodies against CD4 and CD8 and analyzed by flow cytometry. **E.** Purified resting mature T cells from wildtype and IL-7R^{flox} animals were cultured in the presence or absence of IL-7 and analyzed for cell survival by PI exclusion at 24 hour intervals. **F.** and **G.** T cells purified from wildtype and IL-7R^{flox} animals were lysed following isolation and/or cultured in the presence or absence of IL-7 for 18hrs prior to lysis and immunoblotting with the indicated antibodies.

were measured and found to be comparable, indicating that the IL-7R^{flox} transgene did not alter regulation of these proteins (Figure 4.3F).

To further determine the functionality of the IL-7R^{flox} transgene, its capacity to activate IL-7 intracellular signaling pathways was analyzed. It has previously been demonstrated that IL-7 signaling results in the phosphorylation of the transcription factor STAT5, the kinase Akt, and induction of Pim1 (Wofford et al., 2008). Purified T cells from IL-7R^{flox} and wildtype control animals were cultured in the presence or absence IL-7 and analysis of phospho-STAT5 levels indicated that the activation of this transcription factor was not altered between IL-7R^{flox} and wildtype T cells (Figure 4.3G). The IL-7R^{flox} transgene was also capable of inducing a downstream target of STAT5, Pim1, following IL-7 treatment. Additionally, phospho-Akt levels were increased in both wildtype and IL-7R^{flox} cells following culture in IL-7, although to a greater degree in wildtype cells. Together, these data indicate that the IL-7R^{flox} transgene rescued the T cell developmental defect of IL-7R$^{-/-}$ animals, and produced mature T cells that are capable of inducing canonical IL-7 signaling pathways.

4.2.4 Loss of IL-7R decreases size, cell number, and signaling

As the IL-7^{flox} transgene appeared to produce normal T cells, we next investigated the effects of excision of the IL-7R. IL-7R^{flox} transgenic mice were treated with Tamoxifen by intraperitoneal injection to activate Cre recombinase, which would excise the IL-7R transgene *in vivo* to produce IL-7R^{null} cells. Thymocytes and purified mature T cells were examined 3 days post-injection. Thymocyte levels of IL-7R, as determined by flow cytometry, were reduced following Tamoxifen treatment, most markedly in the double positive and CD8 single positive populations (Figure 4.4A). In both CD4+ and CD8+ mature T cells, excision of the IL-7R was on average 80% complete in IL-7R^{null} cells compared to IL-7R^{flox} T cells that did not express the Cre:ER transgene

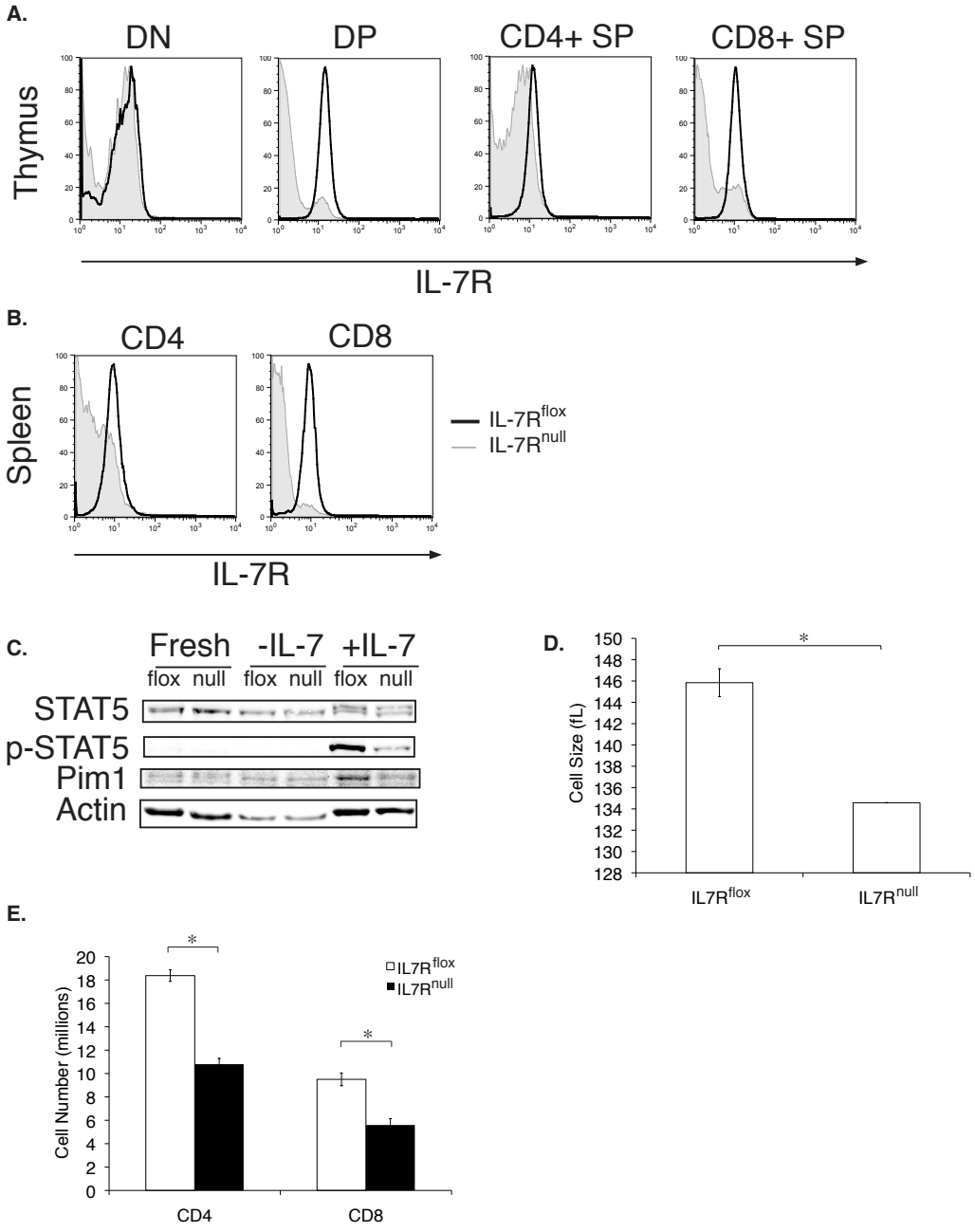

Figure 4.4: The IL-7R is efficiently excised.

IL-7R^{flox} animals both with and without the Cre transgene were treated with Tamoxifen and examined three days after treatment. **A.** and **B.** Thymocytes and splenocytes were stained for CD4, CD8 and the IL-7R and analyzed by flow cytometry. **C.** Following isolation, purified T cells were lysed or cultured for 18 hours in the presence or absence of the cytokine IL-7, lysed, and immunoblotted with the indicated antibodies. **D.** Cell size was measured by particle size analyzer on purified T cells (*, $p < 0.005$). **E.** The number of purified T cells was determined by particle size analyzer and multiplied by the percentage of whole spleen determined to be CD4+ or CD8+ by flow (*, $p < 0.01$).

and also received Tamoxifen (Figure 4.4B). Excision seemed to be more efficient in CD8+ T cells, perhaps suggesting a difference in the regulation of the receptor between CD4+ and CD8+ cells.

To confirm that loss of the IL-7R transgene prevented T cell responses to IL-7, IL-7R^{null} T cells or IL-7R^{flox} purified T cells were cultured in the presence or absence of IL-7. The cytokine was capable of promoting phosphorylation of STAT5 in IL-7R^{flox} T cells, while STAT5 phosphorylation was limited in IL-7R^{null} T cells in the presence of IL-7 (Figure 4.4C). Similarly, Pim1 induction was detectable to a greater extent in IL-7R^{flox} T cells cultured in IL-7 compared to IL-7R^{null} T cells. Excision of the IL-7R also resulted in a significant decrease in the size of IL-7R^{null} resting T cells *ex vivo* compared IL-7R^{flox} (Figure 4.4D), consistent with previous reports indicating that IL-7 maintains cell size *in vitro* (Rathmell et al., 2001). In addition to a decrease in mature T cell size following excision of the IL-7R, the number of CD4+ and CD8+ recovered from spleen and lymph nodes was reduced (Figure 4.4E), suggesting that viability of T cells that fail to receive IL-7R signals *in vivo* is decreased as early as three days post excision.

4.2.5 Loss of IL-7R decreases cell survival

As the number of IL-7R^{null} cells decreased *in vivo* following excision, we next examined the role of IL-7 in mediating survival *in vitro* after loss of the IL-7R. Culture of purified IL-7R^{flox} and IL-7R^{null} T cells in the presence of IL-7 revealed that IL-7R^{flox} T cells exhibited an approximately 70% survival rate during three days of culture while IL-7R^{null} T cells underwent more rapid apoptosis (Figure 4.5A). IL-7R^{null} cells cultured in the absence of cytokine also demonstrated an increased rate of death compared to IL-7R^{flox} cells (Figure 4.5B), suggesting that loss of IL-7R *in vivo* primes cells for a more rapid death. The somewhat greater survival of IL-7R^{null} cells in the presence of IL-7 relative to

IL-7R^{null} cells cultured in the absence of IL-7 was most likely due to the presence of cells in which the receptor did not excise.

The ability of IL-7 to control cell survival may have been due to regulation of Bcl-2 family members. Therefore, expression levels of key Bcl-2 family proteins were analyzed after *in vivo* excision of IL-7R and following overnight culture in the presence or absence of IL-7. In freshly purified T cells, no significant difference was detectable in Mcl-1 or Bcl-2 levels between IL-7R^{flox} and IL-7R^{null} T cells three days after excision *in vivo* (Figure 4.5C). After overnight culture, levels of Bcl-2 and Mcl-1 decreased in IL-7R^{null} cells regardless of the presence of IL-7, while protein levels increased when IL-7R^{flox} cells were cultured in the presence of IL-7. This suggested that the loss of IL-7R resulted in increased cell death and an altered regulation of Bcl-2 family members *in vitro*, however, differences in the Bcl-2 proteins could not be detected *in vivo*, possibly due to rapid death and clearance of cells with decreased Bcl-2 or Mcl-1.

Although the effects of IL-7 signaling on cell survival have been investigated here (Figure 4.1A) and by others (Maraskovsky et al., 1996; Schluns et al., 2000; Tan et al., 2001), much of this work has been carried out in lymphopenic animals or in normal immune systems through injection of IL-7 neutralizing antibodies (Wojciechowski et al., 2007). Although antibody blockade allows for study of IL-7 in a non-lymphopenic immune system, antibodies prevent every cell from receiving an IL-7 signal, and therefore it remained unclear if the observed decrease in cell survival was a T cell intrinsic effect. Our novel inducible system allowed for investigation of IL-7's role in cell survival *in vivo* in the context of a normal immune system. This was accomplished by adoptively transferring naive mature T cells from IL-7R^{flox} animals or wildtype animals into congenic Thy1.1 hosts. All hosts were treated with Tamoxifen, and measurement of the percentage of transferred cells remaining over a two week period indicated that in

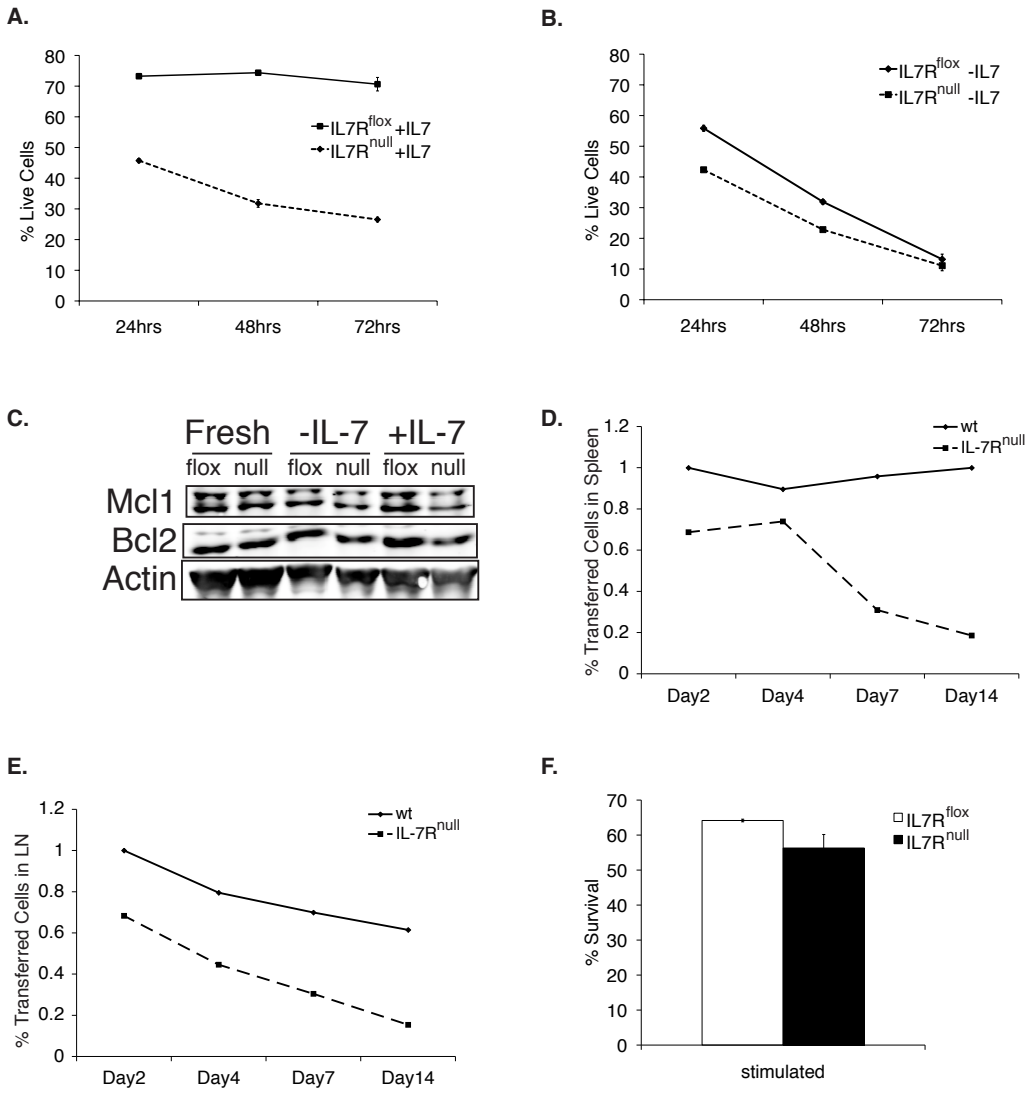

Figure 4.5: Loss of IL-7R signaling effects cell survival.

A., B. and **C.** IL-7R^{flox} animals both with and without the Cre transgene were treated with Tamoxifen, purified three days after treatment, and placed into culture. PI exclusion was used to determine cell survival at 24 hour intervals of cells cultured in the presence of IL-7 (**A**) or absence of IL-7 (**B**). **C.** Freshly purified T cells or those cultured in the presence or absence of IL-7 for 18 hours were lysed and immunoblotted. **D.** and **E.** T cells from IL-7R^{flox} animals or cells from wildtype animals were adoptively transferred in Thy1.1 congenic mice. Twelve hours following transfer, all recipient animals were treated with Tamoxifen and the percentage of transferred cells was determined by staining of splenocyte or lymph node suspension with Thy1.1 and Th1.2 fluorescently conjugated Abs and flow cytometry at the indicated time points. **F.** IL-7R^{flox} and IL-7R^{null} purified T cells were cultured on plates coated with 5μg/mL anti-CD3 and 5μg/mL anti-CD28 antibodies and assayed for cell survival after 2 days by PI exclusion.

both spleen (Figure 4.5D) and intestinal lymph nodes (Figure 4.5E), the percentage of IL-7R^{null} T cells declines more rapidly than wildtype transferred T cells. After two weeks, only 20% of the originally transferred IL-7R^{null} T cells remained in both tissues, whereas wildtype T cell numbers remained constant in the spleen and only decreased 40% in lymph nodes. These data demonstrate that IL-7 is required for naive T cell survival in a normal immune environment in a T cell intrinsic manner.

Although crucial for naive T cells, IL-7 is not thought to be imperative for survival of activated T cells. We predicted that loss of IL-7 signaling would not affect survival of stimulated T cells, and examined this by isolation of IL-7R^{flox} and IL-7R^{null} T cells and culture on plates coated with anti-CD3 and anti-CD28 antibodies. Two days of *in vitro* stimulation revealed that activated IL-7R^{flox} T cells did not demonstrate a significant survival advantage over IL-7R^{null} T cells (Figure 4.5F), further indicating that while IL-7 is required for naive cell survival, it is not necessary for survival of activated T cells.

4.2.6 Growth and proliferation is delayed following loss of the IL-7R

Although IL-7 is not required for T cell survival during stimulation, loss of IL-7 signaling leads to atrophy in naive T cells (Figure 4.4D), and atrophied cells may have a reduced ability to grow rapidly after stimulation. This has been observed in T cells cultured in the absence of cytokine prior to *in vitro* stimulation (Rathmell et al., 2001) and in abnormally developed IL-7R$^{-/-}$ T cells (Maraskovsky et al., 1996). To address the role of IL-7 immediately prior to T cell stimulation, purified IL-7R^{flox} and IL-7R^{null} T cells were cultured on plates coated with anti-CD3 with or without anti-CD28 antibodies for 18 hours prior to measurement of cell size by mean forward scatter. Loss of the IL-7R *in vivo* resulted in a delay in the ability of T cells to increase cell size compared to cells that maintained IL-7 signaling prior to stimulation (Figure 4.6A). Cell size was also measured

by particle size analyzer after 20 hours of stimulation, and both anti-CD3 only and anti-CD3 plus anti-CD28 costimulated cells were smaller while unstimulated cells were not significantly different in size (Figure 4.6B). Loss of IL-7R *in vivo* prior to stimulation also resulted in decreased proliferation as measured by CFSE dilution (Figure 4.6C). Proliferation differences were detectable in T cells stimulated with CD3 alone and CD28 costimulation. These data suggest that while IL-7 does not play a direct role in stimulation, loss of IL-7 signaling and *in vivo* atrophy impair the ability of T cells to grow and proliferate.

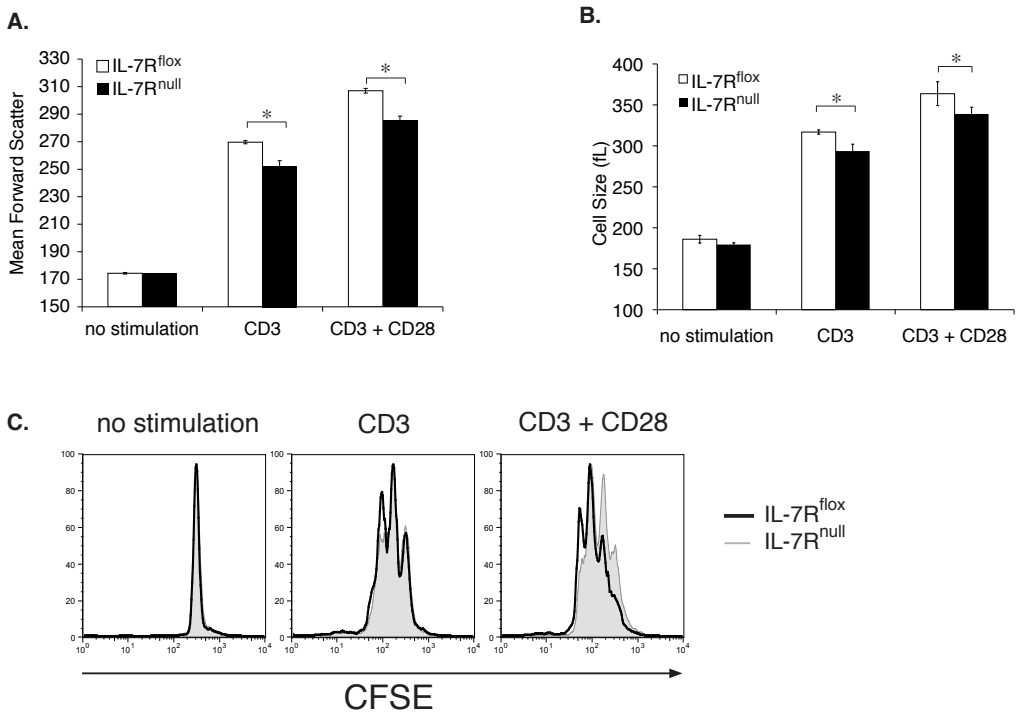

Figure 4.6: Loss of IL-7R results in delayed stimulation.

IL-7R^{flox} and IL-7R^{null} purified T cells were cultured on uncoated or plates coated with 5μg/mL anti-CD3 antibody with or without 5μg/mL anti-CD28 antibody. **A.** Cells were analyzed 18 hours after start of culture for cell size by mean forward scatter with flow cytometry (*, $p < 0.05$). **B**. Cells were analyzed 20 hours after start of culture for cell size by particle size analyzer (*, $p < 0.05$). **C.** Cells were stained with CFSE prior to culture, and analyzed for proliferation by flow cytometry and CFSE depletion after 48 hours.

4.2.7 Glycolysis is regulated by IL-7 signaling *in vivo*

It has been previously demonstrated that glucose metabolism can be regulated by IL-7 *in vitro* and is essential to prevent T cell atrophy *in vivo* (Rathmell et al., 2001; Wofford et al., 2008), and therefore IL-7R may play an essential role in sustaining T cell glucose metabolism *in vivo* to prevent cellular atrophy. To determine the role of IL-7 signaling *in vivo* on glucose metabolism, we first established that the IL-7R^{flox} transgene modulated glucose metabolism in a manner similar to wildtype T cells. First, total levels of Glut1 protein were determined by permeabilization and flow cytometry. Interestingly, IL-7R^{flox} transgenic T cells had decreased levels of total Glut1 protein compared to wildtype T cells (Figure 4.7A), although determination by immunoblotting did not correspond with this result (Figure 4.7B). Regardless, these differences in Glut1 protein levels did not result in a significant change in glucose uptake, as both cell types exhibited a similar level of glucose uptake *ex vivo* and maintenance in glucose uptake in response to IL-7 (Figure 4.7C). Additionally, glycolytic flux was not significantly altered between IL-7R^{flox} transgenic T cells and wildtype cells *ex vivo* (Figure 4.7D). These data demonstrate that the IL-7R^{flox} transgene regulates glucose metabolism in a manner similar to wildtype T cells.

Effects of IL-7R excision, such as delayed activation and reduced survival, may potentially be caused by a reduction in glucose metabolism. Loss of IL-7 signals could affect glucose metabolism at the level of the glucose transporter, rate of glucose uptake, or flux through glycolysis. Unexpectedly, IL-7R^{null} purified T cells exhibited increased total levels of Glut1 protein compared to IL-7R^{flox} T cells (Figure 4.8A and B). However, loss of IL-7R *in vivo* did not seem to affect glucose transport, as *ex vivo* glucose uptake was unchanged in IL-7R^{null} T cells compared to IL-7R^{flox} T cells (Figure 4.8C). Due to the method used to detect glucose uptake, increased Glut1 protein without a change in

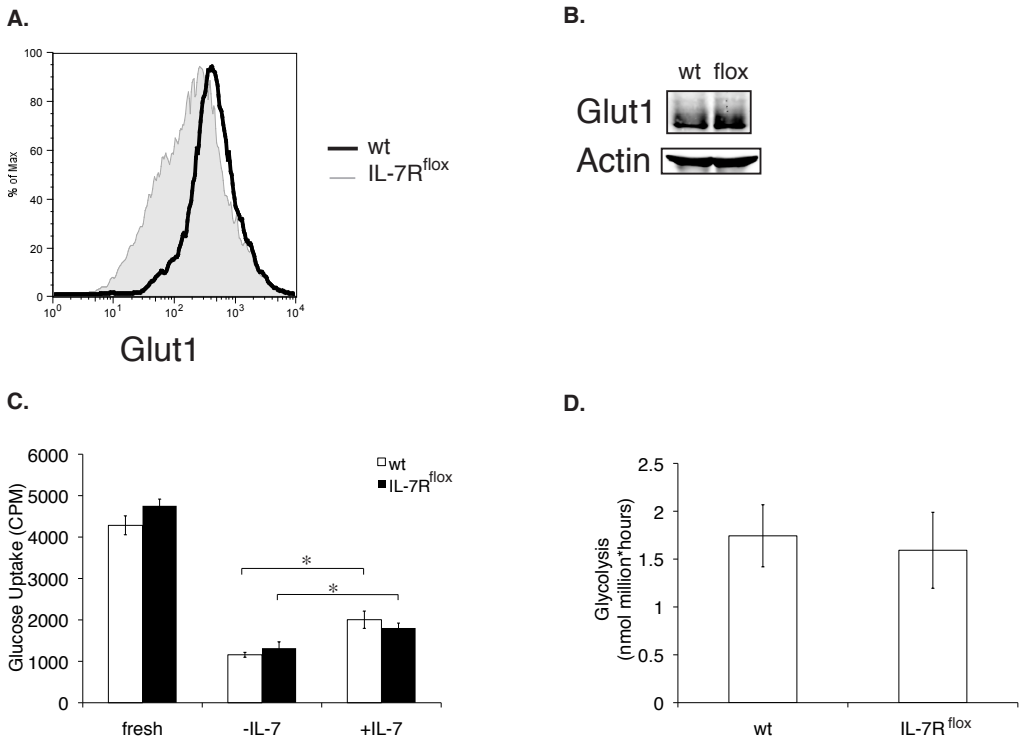

Figure 4.7: IL-7R^{flox} transgenic T cells have metabolic responses similar to wildtype.

A. Purified resting mature T cells from wildtype and IL-7R^{flox} animals were permeabilized and stained with anti-Glut1 antibody to determine total Glut1 protein levels by flow cytometry. **B.** Freshly purified T cells were lysed and immunoblotted for Glut1. **C.** T cells purified from wildtype and IL-7R^{flox} animals were analyzed for ability to uptake glucose from resting T cells or cells cultured in the presence or absence of IL-7 for 24 hours (*, $p < 0.02$). **C.** Purified resting T cells from wildtype and IL-7R^{flox} animals were exposed to ^{3}H-Glucose to measure glycolytic flux.

uptake may be due to alteration in Hexokinase (HK) activity, an enzyme that phosphorylates glucose to generate glucose-6-phosphate, and is the first regulated step of glycolysis. Determination of HK activity of IL-7R^{flox} and IL-7R^{null} T cells indicated that while HK activity appeared lower in IL-7R^{null} T cells, this difference was not significant (Figure 4.8D). As IL-7 can maintain glucose uptake *in vitro*, it was surprising that neither HK activity nor glucose uptake was modified *ex vivo*, and suggested that while IL-7 is an essential requirement for cell survival, the effects of IL-7 signaling loss on glucose uptake may be altered but compensated for by an unknown pathway. Compensation

pathways could increase levels of the Glut1 transporter or HK activity to maintain glucose uptake at normal rates. However, this level of glucose uptake was not capable of maintaining cell survival *in vivo* (Figure 4.5D and E), suggesting that IL-7 may regulate glucose metabolism downstream of glucose uptake, such as during glycolysis. Analysis

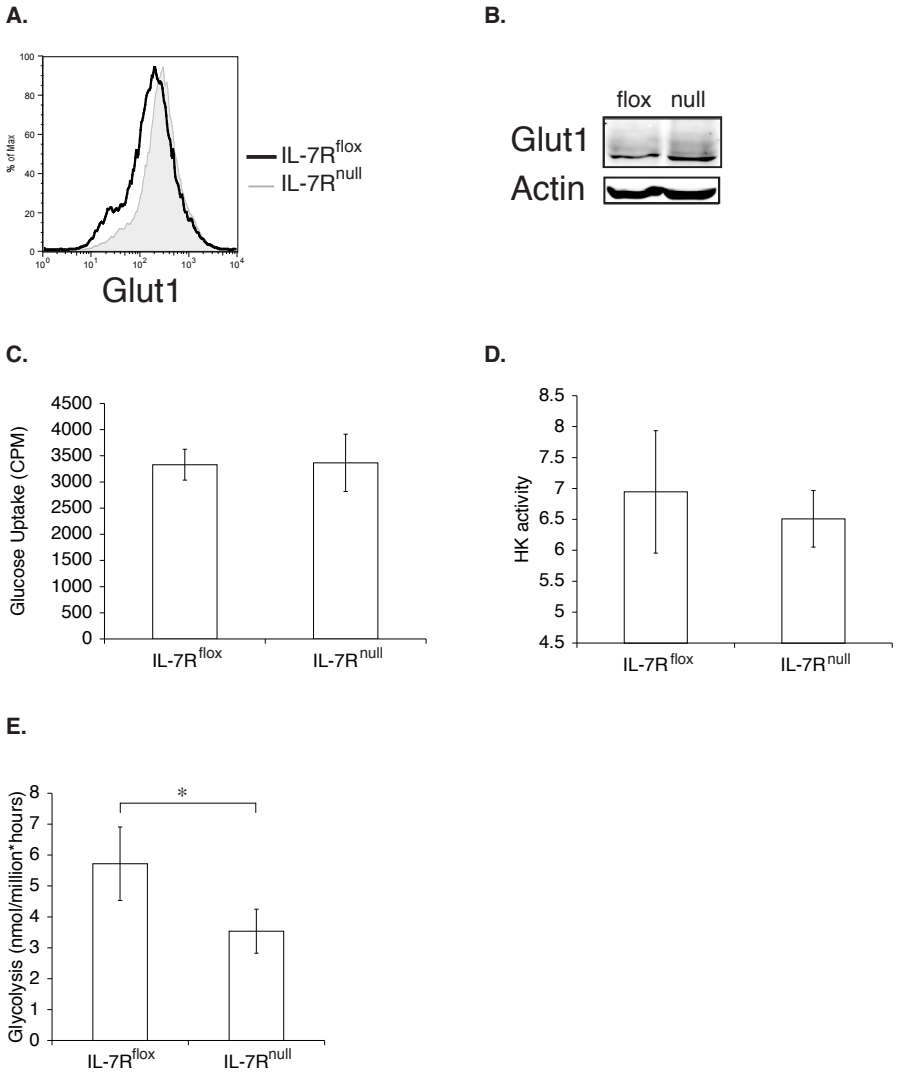

Figure 4.8: Loss of IL-7R reduced glycolysis rate *in vivo*.

A. IL-7R^{flox} and IL-7R^{null} purified T cells were permeabilized and stained with anti-Glut1 antibody to determine total Glut1 protein levels by flow cytometry. **B.** Freshly purified T cells were lysed and immunoblotted for Glut1. **C.** IL-7R^{flox} and IL-7R^{null} purified resting T cells were analyzed for ability to uptake glucose, **D.** HK activity and **E.** glycolytic flux (*, $p < 0.001$).

of glycolytic flux indicated that IL-7R^{null} T cells exhibited decreased glycolytic flux *ex vivo* compared to IL-7R^{flox} T cells (Figure 4.8E). This is a novel finding, and suggests that following the loss of IL-7R, a decrease in glycolytic flux may result in an inability of T cells to produce the energy required to prevent atrophy and maintain survival.

4.3 Discussion

This work demonstrates a novel role for IL-7 as a unique signal to maintain glycolysis of T cells *in vivo,* and suggests that this metabolic regulation may be key for increasing cell survival and priming for growth. Through the development of an inducible IL-7R knockout system, we identified the regulation of glycolysis as a key role for IL-7 and uniquely described the ability of IL-7 to maintain T cell survival in a complete lymphoid compartment. However, the mechanism of cell survival maintenance remains elusive, as levels of Bcl-2 family members appeared unchanged *in vivo*, possibly due to death of cells with decreased levels of protein. Additionally, IL-7 signaling was important prior to T cell activation, as the ability of cells to stimulate was decreased in the absence of IL-7 signaling. Taken together, this work demonstrates the importance of IL-7 signaling for naive T cell homeostasis.

Although IL-7 has been shown to regulate glucose uptake *in vitro*, our model did not demonstrate that IL-7 was critical for maintaining glucose uptake *in vivo*. This may be due to the continued presence of other cell extrinsic signals *in vivo* that are absent *in vitro*. Self-TCR activation was still received by IL-7R^{null} T cells *in vivo*, a signal that has been shown to mediate naive T cell homeostasis (Takeda et al., 1996; Tanchot et al., 1997). Although there has been no evidence that self-antigen TCR signals may regulate glucose uptake, it has been shown that TCR ligation can increase glucose uptake *in vitro* (Frauwirth et al., 2002). This indicates that TCR signals are capable of regulating glucose

uptake and self-TCR ligation may promote uptake *in vivo* even in the absence of IL-7R. A second explanation for maintenance of glucose uptake following loss of the IL-7R may be due to the decrease in glycolysis (Figure 4.8E). This decrease in glycolysis may result in a decrease in cellular energy and result in compensatory pathways within the cell to acquire more energy. It has been previously shown that glucose deprivation increases Glut1 protein levels by increasing RNA stability (Boado and Pardridge, 2002). In support of compensatory increase in glucose uptake, loss of IL-7R signaling resulted in an increase in total Glut1 protein (Figure 4.8A and B). Decreases in intracellular energy levels may also lead to the use of other fuel sources such as glutamine, an important amino acid during lymphocyte activation (Bental and Deutsch, 1993).

In addition to identifying a unique role of IL-7 for regulation of glycolysis *in vivo*, we also demonstrate that IL-7 is required for naive T cell homeostasis in the context of a normal immune system. Initial attempts to determine the role of IL-7 *in vivo* for homeostasis, accomplished by injecting animals with IL-7 blocking antibodies, resulted in blockade of T cell development but did not affect T cell survival (Grabstein et al., 1993). This was most likely due to incomplete blockade of the IL-7 signal, as later experiments with IL-7 neutralizing antibodies demonstrated a role of IL-7 in T cell homeostasis *in vivo* similar to those observed though adoptive transfer experiments (Wojciechowski et al., 2007). Neutralizing antibody experiments demonstrated that IL-7 is capable of regulating T cell homeostasis in a normal immune environment, unlike adoptive transfer into IL-$7^{-/-}$ hosts. However, it does not demonstrate that this effect on cell survival is T cell intrinsic. Here, through the use of an inducible Cre/Lox system, we demonstrate that IL-7 is required for naive T cell homeostasis and that this process is T cell intrinsic (Figure 4.5 D and E). Adoptive transfers, neutralizing antibodies, and inducible excision have all demonstrated a similar half-life of naive T cells survival

without IL-7 signaling of about one week *in vivo*, suggesting that these methods are equivalent.

Although we define a clear role for IL-7 *in vivo* to regulate T cell survival, the specific method of regulation of this survival remains unclear. While Bcl-2 family members Bcl-2 and Mcl-1 have been implicated in IL-7's role to mediate survival, neither is the sole means by which IL-7 allows cells to persist (Akashi et al., 1997; Maraskovsky et al., 1997; Zhou et al., 1998). Surprisingly, excision of the IL-7R did not result in modification of Bcl-2 or Mcl-1 levels *in vivo* (Figure 4.5C). This may have been due to rapid clearance of T cells *in vivo* that have reduced expression levels of these anti-apoptotic factors. The possibility remains, however, that cell survival may be regulated upstream of Bcl-2 family members by another factor, such as glucose metabolism.

IL-7 may maintain T cell homeostasis through the regulation of glucose metabolism as has been reported in various systems. In developing thymocytes, deprivation from Notch signaling decreased glucose uptake and resulted in rapid apoptosis (Ciofani and Zuniga-Pflucker, 2005). Expression of a constitutively active Akt in these cells maintained glucose uptake and replaced Notch signals to prevent cell death. Similarly, constitutively active Akt expression in growth factor dependent hematopoietic cell lines requires glucose to promote cell survival in the absence of growth factor (Plas et al., 2001). Further evidence from growth factor dependent cells has suggested that glucose metabolism can regulate Bcl-2 family members. An increase in glucose metabolism confers a cell survival increase via GSK3-dependent maintenance of Mcl-1 following growth factor withdrawal (Zhao et al., 2007). Withdrawal from glucose, however, resulted in induction of pro-apoptotic protein Puma in a p53 dependent fashion (Zhao et al., 2008). These data indicate that cellular glucose metabolism in

hematopoietic cells is capable of regulating survival, and IL-7 may regulate homeostasis through control of glycolysis via similar signaling pathways.

Understanding the signaling of the IL-7R and how it maintains T cell survival can be useful for the treatment of multiple diseases. New insight into the regulation of T cell pool size can aid patients with immunodeficiency due to spontaneous mutation, radiation, or acquired immunodeficiency. Future study of IL-7 and other cell extrinsic signaling effects on cell survival and glucose metabolism may also led to a better understanding of how cancer cells can survive independent of these signals and acquire an abnormal amount of nutrients from their environment.

5. Conclusions and Future Directions

The work described here outlines a role for the regulation of glucose metabolism in both resting and active T lymphocytes. Glucose is required during T cell immune responses, as T cells increase their glucose uptake to meet the demands associated with increased proliferation and growth. Glucose uptake must be tightly regulated, however, as increased glucose uptake in resting T cells led to high levels of activation and an autoimmune-like phenotype. Cell extrinsic signals were critical for the regulation of glucose metabolism. Costimulatory signals in addition to TCR ligation were required for maximal increases in glucose uptake during T cell activation in part through activation of Akt to promote or maintain Glut1 on the cell surface. Transgenic expression of a constitutively active Akt resulted in an increase in glucose uptake in resting T cells, and consistent with a role of Akt to promote Glut1 surface levels, Glut1/mAkt double transgenic animals displayed a synergistic increase in glucose uptake. Together these data suggest that induction of Glut1 protein and Akt regulation of trafficking are separable functions of CD28 costimulatory signaling that cooperate to promote the required increase in glucose uptake for T cell activation and proliferation.

Glucose uptake increases dramatically during T cell stimulation, in response to cell extrinsic signals through the TCR. Similarly, quiescent T cells also require cell extrinsic signals to maintain glucose metabolism and survival. Through the production of an inducible IL-7R knockout system, we were able to demonstrate the role of this cytokine in the regulation of naive T cell survival in the context of a normal immune environment. Additionally, we demonstrate that IL-7 is capable of maintaining glycolytic flux *in vivo*, and suggest that IL-7 may partially mediate T cell homeostasis through the regulation of glucose metabolism. Together, these data outline the

importance of cell extrinsic signals to mediate not only T cell responses to antigen but to maintain cell survival and that both these signals are capable of regulating glucose metabolism. These studies pose new questions about the regulation of Glut1 protein levels, other signals that may regulate metabolism in naive T cells, and the relationship between glucose and cell survival.

5.1 Induction of Glut1 protein following TCR ligation

We have demonstrated that Glut1 protein levels increase following a strong TCR ligation even without costimulation (Figure 3.2B). While attempts were made with pharmacological inhibitors to determine how Glut1 protein levels are regulated in response to TCR ligation (Figure 3.3), blockade of PI3K, calcineurin, MAPK, and PKC pathways all resulted in decreased glut1 message, Glut1 protein, and glucose uptake, albeit to different degrees. To better determine how Glut1 protein levels are increased (Figure 5.1), an alternate approach may be to examine the Glut1 promoter. As all inhibitors we investigated reduced the amount of glut1 mRNA, it seems likely that TCR induced increases in Glut1 protein are due to an increase in transcription or RNA stability, rather than translational control. To determine if RNA stability of Glut1 is increased by TCR ligation, cells could be treated with actinomycin and measurement of the amount of glut1 mRNA that persists by qRT-PCR at various time points after treatment. If TCR signaling increases glut1 mRNA stability, it could be further investigated through genetic ablation of the ARE element of Glut1 or the RNA binding protein HuR (Gantt et al., 2006; Jain et al., 1997). If loss of these factors prevents TCR induced increases in glut1 mRNA, it is likely that RNA stability is the mechanism of increased Glut1 protein levels. Glut1 may also be transcriptionally controlled and determining what transcription factors are bound to the Glut1 promoter through

footprinting or chromosomal immunoprecipitation following TCR ligation and comparison with unstimulated cells or cells receiving a costimulatory signal may reveal the mechanism of this regulation. This will not only identify the signaling pathways that increase Glut1 levels downstream of the TCR, but may also determine how costimulation affects Glut1 levels. As costimulation results in an increase in glut1 message levels even with low TCR stimulation (Figure 3.2A), it is possible that costimulation may alter the number or kind of transcription factors found on the Glut1 promoter. I hypothesize that many of the transcription factors, such as AP-1, NF-κB, and NFAT, that are known to regulate the transcription of the IL-2 gene during activation will also play a role in the transcription of Glut1 (Crispin and Tsokos, 2009). Data concerning the transcription of IL-2 may serve as a guide for faster identification of transcription factors regulating Glut1.

In addition to TCR-regulated increases in Glut1, other questions remain, such as the nature of the autoimmune-like phenotype in Glut1 transgenic animals. Animals exposed to increased glucose uptake in their T cells for over a year developed hyper-gammaglobulinemia and increased antibody deposition in the kidneys (Figure 3.7). This suggested that glucose uptake higher than that of normal naive T cells may lead to the development of disease. Although these animals presented with an autoimmune-like phenotype, this did not result in shortened lifespan or obvious disease symptoms. To test the role of glucose uptake in specific pathways that lead to autoimmunity, autoimmunity could be directly promoted in specific tissues and pathways in Glut1 transgenic mice. This could be accomplished through induced autoimmunity such as the experimental autoimmune encephalomyelitis model, where injection of myelin basic protein results in eventual paralysis, which can be scored by severity (Martin and McFarland, 1995). Alternatively, Glut1 transgenic mice could be bred to a genetic model

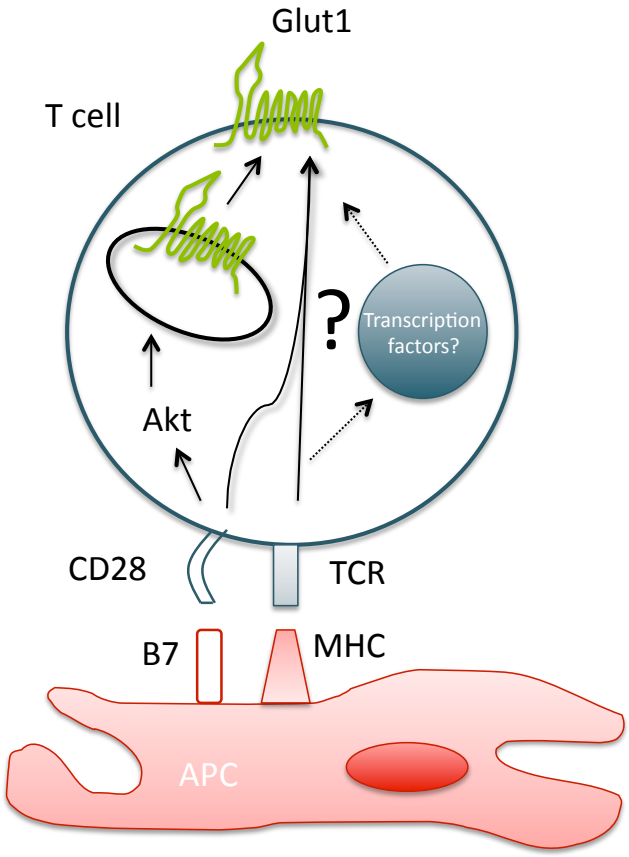

Figure 5.1: How is Glut1 increased downstream of TCR signals?

Data presented in chapter 3 indicates that TCR signals induce Glut1 protein levels, although it is unclear if this increase in Glut1 protein is mediated by transcriptional control or otherwise. Increases in Glut1 protein levels can be augmented by CD28 costimulation. A second costimulation pathway activates Akt to promote localization of Glut1 to the cell surface.

of autoimmunity, such as the MRL-lpr/lpr mouse, which spontaneously develops an autoimmune disease similar to systemic lupus erythematosus (Heber-Katz et al., 2004). It is likely that increased glucose uptake will exacerbate the autoimmune disease in these animals, and may identify specific checkpoints in tolerance that may be affected by increased Glut1 expression. This would further support the importance of tightly regulated glucose uptake to prevent unwanted T cell activation and inflammation.

In addition to contributing to autoimmune diseases, the effects of increased glucose uptake in T cells may be exacerbated by metabolic dysfunction. To this end, it would be of great interest to determine the effects of increased glucose uptake in T cells during induction of diabetes. This could be accomplished by feeding Glut1 transgenic animals a high fat diet (HFD) to induce insulin resistance or by breeding to a diabetes-prone mouse model, such as NOD mice. This would result in hyperglycemia in these animals, and comparison of T cell activation and function in control, diabetic, Glut1, and diabetic/Glut1 animals would determine if increased glucose availability combined with increased glucose uptake exacerbates naive T cell activation. The cause of T cell activation differences may be difficult to determine in NOD animals as diabetes development is dependent on autoimmunity (Anderson and Bluestone, 2005). Furthermore, HFD may alter the nutrient content available to T cells, as well as result in obesity that is often associated with increased inflammation (Duffaut et al., 2009). Not only will these experiments determine the effect of hyperglycemia in T cells with increased glucose uptake, but will determine if Glut1 transgenic T cells exacerbate the progression of diabetes and the systemic inflammation that characterizes the metabolic syndrome.

5.2 Unchanged glucose uptake in IL-7R^{null} T cells

Here we have identified a novel role for IL-7 in controlling the flux of glycolysis *in vivo* and altering the energy available to naive T cells (Figure 4.8E). However, loss of IL-7 signals *in vivo* did not change the rate of glucose uptake (Figure 4.8C), and surprisingly resulted in an increase in Glut1 protein levels (Figure 4.8A and B). This suggests that loss of IL-7 signaling resulted in activation of a compensatory signal capable of regulating glucose metabolism. While paradoxical, a similar finding was

observed in T cells neglected or treated with IL-7 *in vitro* (Wofford et al., 2008). A compensatory signal for altered metabolism likely occurs due to decreased glycolysis and ATP production. Nutrient sensitive signaling pathways may compensate by increasing levels of Glut1 protein, resulting in maintenance of glucose uptake (Figure 5.2). Reduction of ATP or NADPH levels following excision of the IL-7R would indicate that cellular energy stores are being reduced and suggest that compensation is likely. However, it may be difficult to see a change in the levels of these energetic molecules if a compensatory mechanism is maintaining them or cells switch to alternative fuel to sustain energetics. Therefore, a time course may be critical to observe decreases in ATP or NADPH levels. Even if no decrease in levels was identified, activation of energy

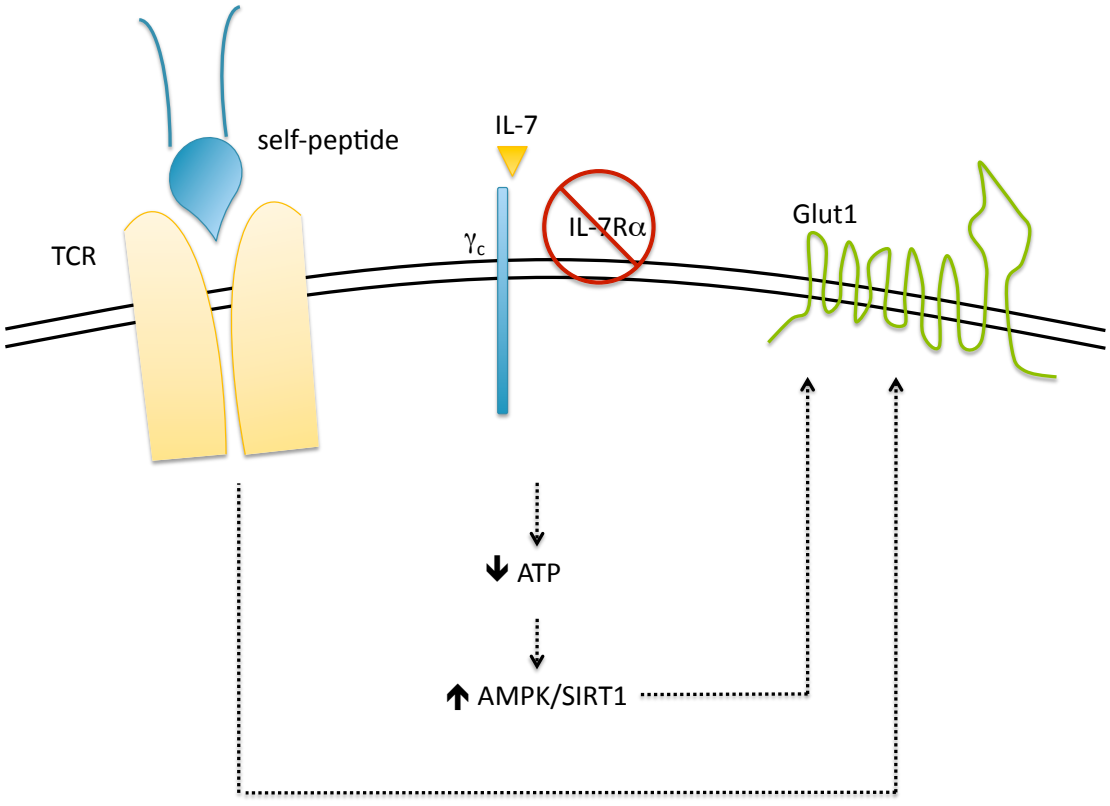

Figure 5.2: Possible compensation signals following the loss of IL-7R

Deletion of the IL-7R may result in an increase in Glut1 protein levels through energetic feedback pathways. Decreased glycolysis may reduce ATP levels and result in activation of nutrient sensors to maintain Glut1. Alternatively, Glut1 protein levels may be regulated by a different cell extrinsic signal such as the TCR.

sensing molecules such as AMPK or SIRT1 when IL-7 signals are removed would suggest that intracellular signaling pathways are maintaining metabolism through a compensatory pathway. AMPK functions as a sensor for cellular energy status and increases ATP production in response to increased AMP levels. Similarly, SIRT1 is a transcription factor that regulates metabolic responses to starvation or stress (Fulco and Sartorelli, 2008). If activation of these proteins is found, genetic ablation of these energy sensors resulting in a decrease in glucose metabolism following excision of the IL-7R would indicate that they are responsible for the compensation effects we have seen here.

Compensation for the loss of IL-7 could also occur external to the cell via a secondary signaling pathway. It is possible that multiple signals may regulate glucose uptake, including IL-7 or self-TCR signals. Like IL-7, weak TCR signals are required for naive T cell homeostasis (Surh and Sprent, 2008). To determine if self-peptide/MHC interactions with the TCR can regulate glucose uptake or metabolism *in vivo*, inducible excision of the TCR (Polic et al., 2001), or of the proteins required for the proximal signaling complex such as LAT (Shen et al., 2009), Lck, or Fyn (Seddon and Zamoyska, 2002), could establish a role for TCR signaling in the regulation of glucose metabolism. Loss of these signals results in gradual death of naive T cells *in vivo*, but the effect of their loss on glucose metabolism has not been examined. It is possible that TCR signals may be the main regulator of glucose uptake, but that it can be augmented by IL-7 signals or that both are able to regulate glucose uptake of T cells equally. To fully investigate the role of these homeostatic signals on naive T cell glucose metabolism, inducible excision models could be combined to ablate both TCR signals and IL-7 signals *in vivo*. This would determine the ability of these two signals to regulate glucose metabolism *in vivo* and their impact on this regulation relative to one another.

Additionally, this would allow for the study of *in vivo* life span of T cells without these two critical signals, and whether loss of both signals would result in decreased *in vivo* survival compared to the loss of either signal alone. Interestingly, if ablation of both IL-7 and TCR signals does not reduce life span compared to the loss of only one signal, this may suggest that there remain addition homeostatic signals *in vivo* yet to be discovered.

The novel Cre/Lox inducible system demonstrated that IL-7 is essential to regulate glycolysis *in vivo*, but how IL-7R mediates this effect is yet to be determined as glycolysis may be regulated at multiple stages. A prime candidate for regulation is Hexokinase, although preliminary examination of Hexokinase activity did not reveal significant loss of activity following excision of the IL-7R (Figure 4.8D). This may, however, vary depending on the level of excision, any possible background inflammation, and time point. To determine how IL-7 may regulate glycolytic flux, other enzymes involved in glycolysis should be examined for changes in activity level, protein level, and message level (Figure 5.3). Activity of glycolytic enzymes following IL-7R loss can be measured by addition of recombinant enzymes above and below them in the glycolytic pathway so that the enzyme in question is rate limiting for the production of NADPH from NADP+. Altered enzyme activity may reflect changes in protein or message levels of these enzymes, which could be assessed by western blot or qRT-PCR. If differences are observed in protein levels, the mechanism for this regulation could be revealed by treatment with cyclohexamide to prohibit new protein production or protease or lysosomal inhibitors to block protein degradation. If changes in protein levels of glycolytic enzymes were observed, this analysis would determine whether IL-7 controls glycolytic flux by encouraging new protein synthesis, or by preventing protein degradation. However, examination of glycolytic enzyme message levels, if altered following excision of the IL-7 receptor, may indicate that IL-7 signaling controls

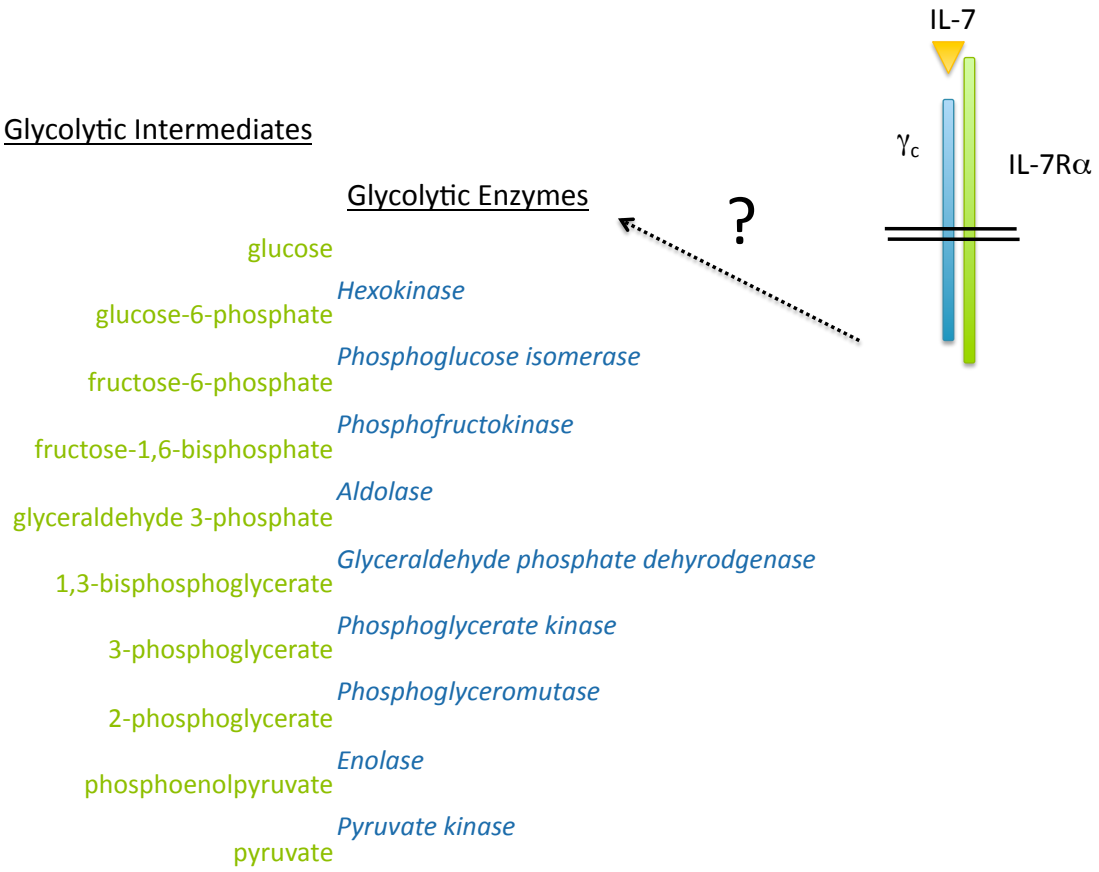

Figure 5.3: IL-7 signaling may regulate glycolytic enzymes.

The IL-7R may regulate glycolysis by affecting the activity or protein level of any of the involved enzymes listed here with their substrate and product.

transcription. I would hypothesize that transcriptional regulation of glycolytic enzymes could be possible through STAT5, a transcription factor known to be regulated by IL-7 signaling (Pallard et al., 1999). If differences in glycolytic message levels are found, the role of STAT5 in this regulation could be examined through the use of STAT5 inducible knockout animals (Cui et al., 2004). If loss of STAT5 results in decreased message levels of glycolytic enzymes, similarly to loss of IL-7R, it is likely that IL-7 maintains glycolytic flux through STAT5 transcriptional regulation of key enzymes.

While the exact mechanism remains unclear, we determined that IL-7 signaling regulates glucose metabolism and homeostasis of naive T cells. However, IL-7 has effects beyond those on naive cells, including roles in lymphopoesis, lymphopenia induced proliferation, memory cell development, and memory cell survival (Jiang et al., 2005). These other functions of IL-7 could be studied in detail with our novel transgenic inducible knockout model. Introduction of transgenic TCRs or the use of the lymphocytic choriomeningitis virus infection model to induce robust immune responses *in vivo* combined with the inducible IL-7R knockout would allow for analysis of the role of IL-7 in the development and maintenance of memory cells. IL-15 has also been implicated in memory cell development, and this system could be used to distinguish the role of IL-7 from this other memory signal (Schluns and Lefrancois, 2003). Additionally, the work outlined here was performed on CD3+ T cells, which could be sorted into multiple different T cell subsets, allowing the relative importance of IL-7 in these types of cells to be determined. It has been established that CD4+ and CD8+ memory T cells have different requirements for IL-7 in lymphopenia-induced proliferation (Tan et al., 2002). Additionally, IL-7 signaling has been implicated in the development of T regulatory (T_{reg}) cells (Mazzucchelli et al., 2008) but is not able to induce T_{reg} suppressor function (Wuest et al., 2008). Furthermore, little information is available concerning the role of IL-7 on the fairly unique subset of highly inflammatory Th17 T cells. These different subsets of T cells and their requirement for IL-7 to control survival and glucose metabolism could be addressed with our model of *in vivo* excision of the IL-7R followed by staining and sorting.

5.3 IL-7 and Bcl-2 family members

Here we demonstrate that loss of IL-7 signaling results in T cell death in a T cell intrinsic manner, although differences in the Bcl-2 family members Bcl-2 or Mcl-1 were not observed in freshly isolated T cells (Figure 4.5C). Decreases in these anti-apoptotic proteins were expected, as it has been previously demonstrated that IL-7 regulates the amount of these proteins in the cell (Jiang et al., 2004). This is mostly likely due to compensation by other growth factors or death of cells in which levels of Bcl-2 or Mcl-1 were reduced. In support of the latter hypothesis, cultures revealed differences in protein levels in the absence of IL-7 compared to cells cultured in the cytokine (Figure 4.5C). Breeding IL-7R inducible knockout animals to Mcl-1 or Bcl-2 transgenic animals, which over express these anti-apoptotic proteins specifically in T cells, would allow for clarification of Bcl-2 family members' role in homeostasis of naive T cells.

Additionally, it has been demonstrated that the ratio of Bim to Bcl-2 is critical for IL-7 regulated maintenance of naive T cells (Wojciechowski et al., 2007). This could be examined in more detail with our inducible IL-7R knockout, and it could be determined if these effects are T cell intrinsic following genetic excision of the receptor, instead of through the use of neutralizing antibodies. This study also brings into question the relative balance of other pro and anti-apoptotic Bcl-2 family members such as Mcl-1 and Bak, which antagonize each other in a manner similar to Bim and Bcl-2 (Willis et al., 2005). Comparison of Mcl-1 conditional knockout animals (Opferman et al., 2003) to Bak knockout animals (Rathmell et al., 2002) and double Bak/Mcl-1 knockouts with or without IL-7R excision could determine if these proteins play a role downstream of IL-7 in maintaining cell survival *in vivo* individually or combined. It is likely that many of the Bcl-2 family members that are expressed in naive T cells play an important role in regulating cell survival.

We show here that IL-7 is required for naive T cell homeostasis and that IL-7 can regulate glycolysis rates *in vivo*, and suggest that there may be a connection between maintenance of glucose metabolism and cell survival, as has been demonstrated in other systems (Jacobs and Rathmell, 2006). If IL-7 regulates cell survival by maintaining glucose metabolism, forced flux through glycolysis may maintain cell survival in the absence of IL-7 signals. To test this hypothesis, IL-7R inducible knockout animals could be bred to animals that over express Glut1 or constitutively active Akt on T cell specific transgenes. If T cells from animals that exhibit increased glucose uptake survive better following excision of the IL-7R than non-transgenic controls, it demonstrates that increased glucose metabolism is capable of maintaining naive T cell survival *in vivo*, and suggests that IL-7 may regulate homeostasis through this pathway. However, defects in glycolysis downstream of glucose uptake in IL-7R^{null} T cells and the pleiotropic effects of Akt may complicate analysis of these experiments. If experimentation reveals a mechanism for IL-7 regulation of glycolysis, such as maintaining the level of a particular glycolytic enzyme, this information could be used to determine if IL-7 controls survival through metabolism. Transgenic expression of this enzyme resulting in cell survival maintenance in the absence of IL-7 signals would most directly show IL-7 control over cell survival through the regulation of glucose metabolism.

5.4 Cancer relevance

T cells require cell extrinsic signals to maintain glucose metabolism and survival, and to mount an immune response. Activation of T cells results in metabolic changes that mimic those seen in cancer cells, as both T cells and transformed cells grow rapidly, increase in cell size, and switch to an aerobic glycolysis program (Vander Heiden et al., 2009). The advantages of studying increased glycolysis in a non-transformed system

were discussed in detail in section 1.1.3. In addition to the parallels in glucose metabolism between activated T cells and cancer cells, stimulated T cells also exhibit some characteristics similar to the hallmarks of cancer. In a key work, Hanahan and Weinberg outlined six attributes exhibited by cancer cells, including insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replication potential, sustained angiogenesis, evading apoptosis, and self-sufficiency in growth signals (Hanahan and Weinberg, 2000). Although not included in this report, deregulated metabolism has also emerged as a key featured shared by cancer cells (Vander Heiden et al., 2009). While activated T cells do not exhibit all of these trademarks, they are self-sufficient in their growth signals, as they create their own IL-2, switch to an aerobic glycolysis program, and proliferate very rapidly. Additionally, activated T cells that are able to evade cell death can result in autoimmune-like phenotypes (Watanabe-Fukunaga et al., 1992). These characteristics of T cells make them an excellent model to determine how these pathways are regulated in normal cells and how their deregulation may result in cancer not only within T cells, but all tumor types.

If T cells become independent from cell extrinsic growth factors, they may be able to grow unregulated and develop into cancers. For example, transgenic expression of IL-7 in hematopoietic cells, to raise the level of the cytokine and increase survival signals, resulted in B and T cell lymphomas and identified IL-7 as a potential oncogene (Rich et al., 1993). Additionally, high levels of IL-7R are found on multiple Hodgkin's lymphoma cell lines, and these cells seem to make their own IL-7 to prevent cell death in an autocrine fashion (Cattaruzza et al., 2009). IL-7 also seems to play a critical role in maintaining the survival of T cell acute lymphoblastic leukemia cells, as they die *in vitro* but can be maintained by co-culture with stromal cells or IL-7 treatment (Barata et al., 2005). A possible therapy for T cell acute lymphoblastic leukemia currently in trials

involves a fusion protein of the catalytic transmembrane domain of diphtheria toxin fused to IL-7 (DAB389 IL-7), which would result in the death of cells expressing the IL-7R (Sweeney et al., 1998). While this treatment will kill naive T cells as well as cancerous T cells, it may be less evasive than chemotherapy or other standard treatment. Although IL-7 promotion of unregulated T cell growth may induce liquid tumor formation, IL-7 induction of T cells may also increase their anti-tumor activity. In xenograft models, tumor cells that expressed IL-7 were rejected by a CD4+ dependent response, suggesting that high local IL-7 at the tumor site increases anti-tumor immunity (Hock et al., 1991). IL-7 has also been shown to increase the efficacy of anti-cancer vaccines (Pellegrini et al., 2009). These data suggest that IL-7 is capable of playing dual roles in relation to cancers depending on the tumor cell expression of the IL-7R: if tumor cells are not able to bind to IL-7, exogenous administration may increase anti-cancer T cell activity. However, if cancer cells express the IL-7R, IL-7 will increase tumor cell growth.

5.5 Final Remarks

The work reported here focused on glucose as a specific nutrient for lymphocytes, and relates to cancer cells, which increase their usage of this energy rich molecule. However, questions remain about the role of other nutrients, such as amino acids, lipids, and vitamins. For example, glioblastoma cell (transformed with Akt) dependence on glucose can be eliminated by activation of β-oxidation, suggesting that energy from fats was able to substitute for the loss of energy from glucose (Buzzai et al., 2005). Although aerobic glycolysis has been described in both T cells and cancer cells, why cells switch to this rather inefficient energy use program remains elusive. It has been suggested that lymphocytes may not be able to increase oxidative metabolism to a

level sufficient to provide the energy required during activation, or alternatively that stimulation induces more glucose transport into the cell than is required and, as the energy is not needed, it is converted into lactate (Frauwirth and Thompson, 2004).

The energetic needs of individual cells are met ultimately by diet, and the unique connections between diet, cancer, and autoimmune disease are broad questions remaining for future research. Data suggest that there are links between energy intake through diet and immunity, such as dietary supplementation resulting in faster infection clearance in the elderly (Langkamp-Henken et al., 2004). Conversely, calorie restriction in mice leads to a decrease in thymocytes and lymphoma-susceptible subpopulations (Poetschke et al., 2000). Calorie restriction also attenuated development of multiple sclerosis in a mouse model (Piccio et al., 2008), and reduced incidence of spontaneous cancer formation (Kritchevsky, 2001). This evidence suggests a link between available energy, metabolic rate, immune function, and cancer, and raises questions: can cancers be prevented or cured by starvation, can autoimmunity be prevented by limiting glucose availability, and will increased nutrient intake reduce infection and immunodeficiency?

While these global regulation questions remain, and may be difficult to address, it has been useful to study lymphocytes as a model for the regulation of glucose metabolism. This work may be used to investigate metabolic deregulation of cancers by studying cell extrinsic and intrinsic signals that regulate glucose metabolism in other cell types prone to cancers, such as lung, brain, prostate, or breast tissue. This may identify the pathways defined here as being universal or tissue type specific. If the signals that regulate glucose metabolism are variable between cell types, this could lead to an ability to block glucose metabolism in specific cell types and lead to targeted, individualized cancer therapies.

References

Acuto, O., and F. Michel. 2003. CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nat Rev Immunol*. 3:939-51.

Acuto, O., S. Mise-Omata, G. Mangino, and F. Michel. 2003. Molecular modifiers of T cell antigen receptor triggering threshold: the mechanism of CD28 costimulatory receptor. *Immunol Rev*. 192:21-31.

Ahmed, N., and M.V. Berridge. 1999. N-glycosylation of glucose transporter-1 (Glut-1) is associated with increased transporter affinity for glucose in human leukemic cells. *Leuk Res*. 23:395-401.

Akashi, K., M. Kondo, U. von Freeden-Jeffry, R. Murray, and I.L. Weissman. 1997. Bcl-2 rescues T lymphopoiesis in interleukin-7 receptor-deficient mice. *Cell*. 89:1033-41.

Altman, B.J., J.A. Wofford, Y. Zhao, J.L. Coloff, E.C. Ferguson, H.L. Wieman, A.E. Day, O. Ilkayeva, and J.C. Rathmell. 2009. Autophagy provides nutrients but can lead to chop-dependent induction of Bim to sensitize growth factor-deprived cells to apoptosis. *Mol Biol Cell*. 20:1180-91.

Alves, N.L., I.A. Derks, E. Berk, R. Spijker, R.A. van Lier, and E. Eldering. 2006. The Noxa/Mcl-1 axis regulates susceptibility to apoptosis under glucose limitation in dividing T cells. *Immunity*. 24:703-16.

Anderson, M.S., and J.A. Bluestone. 2005. The NOD mouse: a model of immune dysregulation. *Annu Rev Immunol*. 23:447-85.

Angel, P., M. Imagawa, R. Chiu, B. Stein, R.J. Imbra, H.J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin. 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. *Cell*. 49:729-39.

Asano, T., H. Katagiri, K. Takata, J.L. Lin, H. Ishihara, K. Inukai, K. Tsukuda, M. Kikuchi, H. Hirano, Y. Yazaki, and et al. 1991. The role of N-glycosylation of GLUT1 for glucose transport activity. *J Biol Chem*. 266:24632-6.

Asano, T., K. Takata, H. Katagiri, H. Ishihara, K. Inukai, M. Anai, H. Hirano, Y. Yazaki, and Y. Oka. 1993. The role of N-glycosylation in the targeting and stability of GLUT1 glucose transporter. *FEBS Lett*. 324:258-61.

August, A., S. Gibson, Y. Kawakami, T. Kawakami, G.B. Mills, and B. Dupont. 1994. CD28 is associated with and induces the immediate tyrosine phosphorylation

and activation of the Tec family kinase ITK/EMT in the human Jurkat leukemic T-cell line. *Proc Natl Acad Sci U S A*. 91:9347-51.

Baird, A.M., D.C. Thomis, and L.J. Berg. 1998. T cell development and activation in Jak3-deficient mice. *J Leukoc Biol*. 63:669-77.

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.

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.

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.

Barthel, A., S.T. Okino, J. Liao, K. Nakatani, J. Li, J.P. Whitlock, Jr., and R.A. Roth. 1999. Regulation of GLUT1 gene transcription by the serine/threonine kinase Akt1. *J Biol Chem*. 274:20281-6.

Beals, C.R., C.M. Sheridan, C.W. Turck, P. Gardner, and G.R. Crabtree. 1997. Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. *Science*. 275:1930-4.

Bental, M., and C. Deutsch. 1993. Metabolic changes in activated T cells: an NMR study of human peripheral blood lymphocytes. *Magn Reson Med*. 29:317-26.

Bentley, J., D. Itchayanan, K. Barnes, E. McIntosh, X. Tang, C.P. Downes, G.D. Holman, A.D. Whetton, P.J. Owen-Lynch, and S.A. Baldwin. 2003. Interleukin-3-mediated cell survival signals include phosphatidylinositol 3-kinase-dependent translocation of the glucose transporter GLUT1 to the cell surface. *J Biol Chem*. 278:39337-48.

Berg, L.J., L.D. Finkelstein, J.A. Lucas, and P.L. Schwartzberg. 2005. Tec family kinases in T lymphocyte development and function. *Annu Rev Immunol*. 23:549-600.

Boado, R.J., and W.M. Pardridge. 2002. Glucose deprivation and hypoxia increase the expression of the GLUT1 glucose transporter via a specific mRNA cis-acting regulatory element. *J Neurochem*. 80:552-4.

Boise, L.H., A.J. Minn, P.J. Noel, C.H. June, M.A. Accavitti, T. Lindsten, and C.B. Thompson. 1995. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity*. 3:87-98.

Bolotin, E., G. Annett, R. Parkman, and K. Weinberg. 1999. Serum levels of IL-7 in bone marrow transplant recipients: relationship to clinical characteristics and lymphocyte count. *Bone Marrow Transplant*. 23:783-8.

Bouillet, P., S. Cory, L.C. Zhang, A. Strasser, and J.M. Adams. 2001. Degenerative disorders caused by Bcl-2 deficiency prevented by loss of its BH3-only antagonist Bim. *Dev Cell*. 1:645-53.

Boxer, R.B., D.B. Stairs, K.D. Dugan, K.L. Notarfrancesco, C.P. Portocarrero, B.A. Keister, G.K. Belka, H. Cho, J.C. Rathmell, C.B. Thompson, M.J. Birnbaum, and L.A. Chodosh. 2006. Isoform-specific requirement for Akt1 in the developmental regulation of cellular metabolism during lactation. *Cell Metab*. 4:475-90.

Brand, K.A., and U. Hermfisse. 1997. Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. *FASEB J*. 11:388-95.

Bretscher, P., and M. Cohn. 1970. A theory of self-nonself discrimination. *Science*. 169:1042-9.

Brockmann, K. 2009. The expanding phenotype of GLUT1-deficiency syndrome. *Brain Dev*.

Broome, H.E., C.M. Dargan, S. Krajewski, and J.C. Reed. 1995. Expression of Bcl-2, Bcl-x, and Bax after T cell activation and IL-2 withdrawal. *J Immunol*. 155:2311-7.

Buller, C.L., R.D. Loberg, M.H. Fan, Q. Zhu, J.L. Park, E. Vesely, K. Inoki, K.L. Guan, and F.C. Brosius, 3rd. 2008. A GSK-3/TSC2/mTOR pathway regulates glucose uptake and GLUT1 glucose transporter expression. *Am J Physiol Cell Physiol*. 295:C836-43.

Bunn, R.C., M.A. Jensen, and B.C. Reed. 1999. Protein interactions with the glucose transporter binding protein GLUT1CBP that provide a link between GLUT1 and the cytoskeleton. *Mol Biol Cell*. 10:819-32.

Burr, J.S., N.D. Savage, G.E. Messah, S.L. Kimzey, A.S. Shaw, R.H. Arch, and J.M. Green. 2001. Cutting edge: distinct motifs within CD28 regulate T cell proliferation and induction of Bcl-XL. *J Immunol*. 166:5331-5.

Buttgereit, F., G.R. Burmester, and M.D. Brand. 2000. Bioenergetics of immune functions: fundamental and therapeutic aspects. *Immunol Today*. 21:192-9.

Buzzai, M., D.E. Bauer, R.G. Jones, R.J. Deberardinis, G. Hatzivassiliou, R.L. Elstrom, and C.B. Thompson. 2005. The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. *Oncogene*. 24:4165-73.

Cantrell, D. 2002. Protein kinase B (Akt) regulation and function in T lymphocytes. *Semin Immunol*. 14:19-26.

Cattaruzza, L., A. Gloghini, K. Olivo, R. Di Francia, D. Lorenzon, R. De Filippi, A. Carbone, A. Colombatti, A. Pinto, and D. Aldinucci. 2009. Functional coexpression of Interleukin (IL)-7 and its receptor (IL-7R) on Hodgkin and Reed-Sternberg cells: Involvement of IL-7 in tumor cell growth and microenvironmental interactions of Hodgkin's lymphoma. *Int J Cancer*.

Chakrabarti, R., C.Y. Jung, T.P. Lee, H. Liu, and B.K. Mookerjee. 1994. Changes in glucose transport and transporter isoforms during the activation of human peripheral blood lymphocytes by phytohemagglutinin. *J Immunol*. 152:2660-8.

Cham, C.M., and T.F. Gajewski. 2005. Glucose availability regulates IFN-gamma production and p70S6 kinase activation in CD8+ effector T cells. *J Immunol*. 174:4670-7.

Chan, J.Y., S.K. Kong, Y.M. Choy, C.Y. Lee, and K.P. Fung. 1999. Inhibition of glucose transporter gene expression by antisense nucleic acids in HL-60 leukemia cells. *Life Sci*. 65:63-70.

Chang, M., J.A. Hamilton, G.M. Scholz, P. Masendycz, S.L. Macaulay, and C.L. Elsegood. 2009. Phosphatidylinostitol-3 kinase and phospholipase C enhance CSF-1-dependent macrophage survival by controlling glucose uptake. *Cell Signal*.

Chi, M.M., J. Pingsterhaus, M. Carayannopoulos, and K.H. Moley. 2000. Decreased glucose transporter expression triggers BAX-dependent apoptosis in the murine blastocyst. *J Biol Chem*. 275:40252-7.

Christofk, H.R., M.G. Vander Heiden, M.H. Harris, A. Ramanathan, R.E. Gerszten, R. Wei, M.D. Fleming, S.L. Schreiber, and L.C. Cantley. 2008. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature*. 452:230-3.

Ciofani, M., and J.C. Zuniga-Pflucker. 2005. Notch promotes survival of pre-T cells at the beta-selection checkpoint by regulating cellular metabolism. *Nat Immunol*. 6:881-8.

Costello, P.S., A.E. Walters, P.J. Mee, M. Turner, L.F. Reynolds, A. Prisco, N. Sarner, R. Zamoyska, and V.L. Tybulewicz. 1999. The Rho-family GTP exchange factor Vav

is a critical transducer of T cell receptor signals to the calcium, ERK, and NF-kappaB pathways. *Proc Natl Acad Sci U S A*. 96:3035-40.

Crespo, P., K.E. Schuebel, A.A. Ostrom, J.S. Gutkind, and X.R. Bustelo. 1997. Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature*. 385:169-72.

Crispin, J.C., and G.C. Tsokos. 2009. Transcriptional regulation of IL-2 in health and autoimmunity. *Autoimmun Rev*. 8:190-5.

Cui, Y., G. Riedlinger, K. Miyoshi, W. Tang, C. Li, C.X. Deng, G.W. Robinson, and L. Hennighausen. 2004. Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. *Mol Cell Biol*. 24:8037-47.

Deprez, J., D. Vertommen, D.R. Alessi, L. Hue, and M.H. Rider. 1997. Phosphorylation and activation of heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin signaling cascades. *J Biol Chem*. 272:17269-75.

Dhein, J., H. Walczak, C. Baumler, K.M. Debatin, and P.H. Krammer. 1995. Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature*. 373:438-41.

Diehn, M., A.A. Alizadeh, O.J. Rando, C.L. Liu, K. Stankunas, D. Botstein, G.R. Crabtree, and P.O. Brown. 2002. Genomic expression programs and the integration of the CD28 costimulatory signal in T cell activation. *Proc Natl Acad Sci U S A*. 99:11796-801.

Doughty, C.A., B.F. Bleiman, D.J. Wagner, F.J. Dufort, J.M. Mataraza, M.F. Roberts, and T.C. Chiles. 2006. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. *Blood*. 107:4458-65.

Duffaut, C., J. Galitzky, M. Lafontan, and A. Bouloumie. 2009. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. *Biochem Biophys Res Commun*. 384:482-5.

Dugani, C.B., and A. Klip. 2005. Glucose transporter 4: cycling, compartments and controversies. *EMBO Rep*. 6:1137-42.

Durbin, J.E., R. Hackenmiller, M.C. Simon, and D.E. Levy. 1996. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. *Cell*. 84:443-50.

Dustin, M.L. 2009. The cellular context of T cell signaling. *Immunity*. 30:482-92.

Dynan, W.S., and R. Tjian. 1985. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. *Nature*. 316:774-8.

Edinger, A.L., R.M. Cinalli, and C.B. Thompson. 2003. Rab7 prevents growth factor-independent survival by inhibiting cell-autonomous nutrient transporter expression. *Dev Cell*. 5:571-82.

Edinger, A.L., and C.B. Thompson. 2002. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. *Mol Biol Cell*. 13:2276-88.

Elstrom, R.L., D.E. Bauer, M. Buzzai, R. Karnauskas, M.H. Harris, D.R. Plas, H. Zhuang, R.M. Cinalli, A. Alavi, C.M. Rudin, and C.B. Thompson. 2004. Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res*. 64:3892-9.

Eyster, C.A., J.D. Higginson, R. Huebner, N. Porat-Shliom, R. Weigert, W.W. Wu, R.F. Shen, and J.G. Donaldson. 2009. Discovery of New Cargo Proteins that Enter Cells through Clathrin-Independent Endocytosis. *Traffic*.

Faltynek, C.R., S. Wang, D. Miller, E. Young, L. Tiberio, K. Kross, M. Kelley, and E. Kloszewski. 1992. Administration of human recombinant IL-7 to normal and irradiated mice increases the numbers of lymphocytes and some immature cells of the myeloid lineage. *J Immunol*. 149:1276-82.

Fandos, C., M. Sanchez-Feutrie, T. Santalucia, F. Vinals, J. Cadefau, A. Guma, R. Cusso, P. Kaliman, J. Canicio, M. Palacin, and A. Zorzano. 1999. GLUT1 glucose transporter gene transcription is repressed by Sp3. Evidence for a regulatory role of Sp3 during myogenesis. *J Mol Biol*. 294:103-19.

Feugeas, J.P., D. Neel, Y. Goussault, and C. Derappe. 1991. Glycosylation of the human erythrocyte glucose transporter: a minimum structure is required for glucose transport activity. *Biochim Biophys Acta*. 1066:59-62.

Fischer, K.D., Y.Y. Kong, H. Nishina, K. Tedford, L.E. Marengere, I. Kozieradzki, T. Sasaki, M. Starr, G. Chan, S. Gardener, M.P. Nghiem, D. Bouchard, M. Barbacid, A. Bernstein, and J.M. Penninger. 1998. Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. *Curr Biol*. 8:554-62.

Fox, C.J., P.S. Hammerman, R.M. Cinalli, S.R. Master, L.A. Chodosh, and C.B. Thompson. 2003. The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. *Genes Dev*. 17:1841-54.

Fox, C.J., P.S. Hammerman, and C.B. Thompson. 2005. Fuel feeds function: energy metabolism and the T-cell response. *Nat Rev Immunol*. 5:844-52.

Frauwirth, K.A., J.L. Riley, M.H. Harris, R.V. Parry, J.C. Rathmell, D.R. Plas, R.L. Elstrom, C.H. June, and C.B. Thompson. 2002. The CD28 signaling pathway regulates glucose metabolism. *Immunity*. 16:769-77.

Frauwirth, K.A., and C.B. Thompson. 2004. Regulation of T lymphocyte metabolism. *J Immunol*. 172:4661-5.

Fulco, M., and V. Sartorelli. 2008. Comparing and contrasting the roles of AMPK and SIRT1 in metabolic tissues. *Cell Cycle*. 7:3669-79.

Gambhir, S.S. 2002. Molecular imaging of cancer with positron emission tomography. *Nat Rev Cancer*. 2:683-93.

Gantt, K.R., J. Cherry, M. Richardson, V. Karschner, U. Atasoy, and P.H. Pekala. 2006. The regulation of glucose transporter (GLUT1) expression by the RNA binding protein HuR. *J Cell Biochem*. 99:565-74.

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.

Giorgino, F., O. de Robertis, L. Laviola, C. Montrone, S. Perrini, K.C. McCowen, and R.J. Smith. 2000. The sentrin-conjugating enzyme mUbc9 interacts with GLUT4 and GLUT1 glucose transporters and regulates transporter levels in skeletal muscle cells. *Proc Natl Acad Sci U S A*. 97:1125-30.

Grabstein, K.H., T.J. Waldschmidt, F.D. Finkelman, B.W. Hess, A.R. Alpert, N.E. Boiani, A.E. Namen, and P.J. Morrissey. 1993. Inhibition of murine B and T lymphopoiesis in vivo by an anti-interleukin 7 monoclonal antibody. *J Exp Med*. 178:257-64.

Greiner, E.F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem*. 269:31484-90.

Guenther, G.G., E.R. Peralta, K.R. Rosales, S.Y. Wong, L.J. Siskind, and A.L. Edinger. 2008. Ceramide starves cells to death by downregulating nutrient transporter proteins. *Proc Natl Acad Sci U S A*. 105:17402-7.

Han, J., B. Das, W. Wei, L. Van Aelst, R.D. Mosteller, R. Khosravi-Far, J.K. Westwick, C.J. Der, and D. Broek. 1997. Lck regulates Vav activation of members of the Rho family of GTPases. *Mol Cell Biol*. 17:1346-53.

Hanahan, D., and R.A. Weinberg. 2000. The hallmarks of cancer. *Cell*. 100:57-70.

Heber-Katz, E., J. Leferovich, K. Bedelbaeva, D. Gourevitch, and L. Clark. 2004. The scarless heart and the MRL mouse. *Philos Trans R Soc Lond B Biol Sci*. 359:785-93.

Heilig, C.W., T. Saunders, F.C. Brosius, 3rd, K. Moley, K. Heilig, R. Baggs, L. Guo, and D. Conner. 2003. Glucose transporter-1-deficient mice exhibit impaired development and deformities that are similar to diabetic embryopathy. *Proc Natl Acad Sci U S A*. 100:15613-8.

Hellerstein, M., M.B. Hanley, D. Cesar, S. Siler, C. Papageorgopoulos, E. Wieder, D. Schmidt, R. Hoh, R. Neese, D. Macallan, S. Deeks, and J.M. McCune. 1999. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. *Nat Med*. 5:83-9.

Hernandez-Caselles, T., M. Martinez-Esparza, D. Sancho, G. Rubio, and P. Aparicio. 1995. Interleukin-7 rescues human activated T lymphocytes from apoptosis induced by glucocorticoesteroids and regulates bcl-2 and CD25 expression. *Hum Immunol*. 43:181-9.

Hock, H., M. Dorsch, T. Diamantstein, and T. Blankenstein. 1991. Interleukin 7 induces CD4+ T cell-dependent tumor rejection. *J Exp Med*. 174:1291-8.

Holsinger, L.J., I.A. Graef, W. Swat, T. Chi, D.M. Bautista, L. Davidson, R.S. Lewis, F.W. Alt, and G.R. Crabtree. 1998. Defects in actin-cap formation in Vav-deficient mice implicate an actin requirement for lymphocyte signal transduction. *Curr Biol*. 8:563-72.

Hume, D.A., J.L. Radik, E. Ferber, and M.J. Weidemann. 1978. Aerobic glycolysis and lymphocyte transformation. *Biochem J*. 174:703-9.

Jacobs, H., P. Krimpenfort, M. Haks, J. Allen, B. Blom, C. Demolliere, A. Kruisbeek, H. Spits, and A. Berns. 1999. PIM1 reconstitutes thymus cellularity in interleukin 7- and common gamma chain-mutant mice and permits thymocyte maturation in Rag- but not CD3gamma-deficient mice. *J Exp Med*. 190:1059-68.

Jacobs, S.R., and J.C. Rathmell. 2006. Lymphocyte selection by starvation: glucose metabolism and cell death. *Trends Immunol*. 27:4-7.

Jain, R.G., L.G. Andrews, K.M. McGowan, P.H. Pekala, and J.D. Keene. 1997. Ectopic expression of Hel-N1, an RNA-binding protein, increases glucose transporter (GLUT1) expression in 3T3-L1 adipocytes. *Mol Cell Biol*. 17:954-62.

Jameson, S.C. 2002. Maintaining the norm: T-cell homeostasis. *Nat Rev Immunol*. 2:547-56.

Jameson, S.C., K.A. Hogquist, and M.J. Bevan. 1995. Positive selection of thymocytes. *Annu Rev Immunol*. 13:93-126.

Janumyan, Y.M., C.G. Sansam, A. Chattopadhyay, N. Cheng, E.L. Soucie, L.Z. Penn, D. Andrews, C.M. Knudson, and E. Yang. 2003. Bcl-xL/Bcl-2 coordinately regulates apoptosis, cell cycle arrest and cell cycle entry. *Embo J*. 22:5459-70.

Jenkins, M.K., and R.H. Schwartz. 1987. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med*. 165:302-19.

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

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.

Jones, R.G., D.R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M.J. Birnbaum, and C.B. Thompson. 2005. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Mol Cell*. 18:283-93.

Joost, H.G., G.I. Bell, J.D. Best, M.J. Birnbaum, M.J. Charron, Y.T. Chen, H. Doege, D.E. James, H.F. Lodish, K.H. Moley, J.F. Moley, M. Mueckler, S. Rogers, A. Schurmann, S. Seino, and B. Thorens. 2002. Nomenclature of the GLUT/SLC2A family of sugar/polyol transport facilitators. *Am J Physiol Endocrinol Metab*. 282:E974-6.

Jope, R.S., and G.V. Johnson. 2004. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci*. 29:95-102.

June, C.H., J.A. Ledbetter, P.S. Linsley, and C.B. Thompson. 1990. Role of the CD28 receptor in T-cell activation. *Immunol Today*. 11:211-6.

Juntilla, M.M., J.A. Wofford, M.J. Birnbaum, J.C. Rathmell, and G.A. Koretzky. 2007. Akt1 and Akt2 are required for alphabeta thymocyte survival and differentiation. *Proc Natl Acad Sci U S A*. 104:12105-10.

Kan, O., S.A. Baldwin, and A.D. Whetton. 1994. Apoptosis is regulated by the rate of glucose transport in an interleukin 3 dependent cell line. *J Exp Med*. 180:917-23.

Khaled, A.R., and S.K. Durum. 2003. Death and Baxes: mechanisms of lymphotrophic cytokines. *Immunol Rev*. 193:48-57.

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

Kim, J.W., and C.V. Dang. 2005. Multifaceted roles of glycolytic enzymes. *Trends Biochem Sci*. 30:142-50.

Kim, K., C.K. Lee, T.J. Sayers, K. Muegge, and S.K. Durum. 1998. The trophic action of IL-7 on pro-T cells: inhibition of apoptosis of pro-T1, -T2, and -T3 cells correlates with Bcl-2 and Bax levels and is independent of Fas and p53 pathways. *J Immunol*. 160:5735-41.

Komschlies, K.L., T.A. Gregorio, M.E. Gruys, T.C. Back, C.R. Faltynek, and R.H. Wiltrout. 1994. Administration of recombinant human IL-7 to mice alters the composition of B-lineage cells and T cell subsets, enhances T cell function, and induces regression of established metastases. *J Immunol*. 152:5776-84.

Kondo, M., K. Akashi, J. Domen, K. Sugamura, and I.L. Weissman. 1997a. Bcl-2 rescues T lymphopoiesis, but not B or NK cell development, in common gamma chain-deficient mice. *Immunity*. 7:155-62.

Kondo, M., I.L. Weissman, and K. Akashi. 1997b. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell*. 91:661-72.

Koretzky, G.A., F. Abtahian, and M.A. Silverman. 2006. SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat Rev Immunol*. 6:67-78.

Krauss, S., M.D. Brand, and F. Buttgereit. 2001. Signaling takes a breath--new quantitative perspectives on bioenergetics and signal transduction. *Immunity*. 15:497-502.

Krawczyk, C., A. Oliveira-dos-Santos, T. Sasaki, E. Griffiths, P.S. Ohashi, S. Snapper, F. Alt, and J.M. Penninger. 2002. Vav1 controls integrin clustering and MHC/peptide-specific cell adhesion to antigen-presenting cells. *Immunity*. 16:331-43.

Kritchevsky, D. 2001. Caloric restriction and cancer. *J Nutr Sci Vitaminol (Tokyo)*. 47:13-9.

Labrecque, N., L.S. Whitfield, R. Obst, C. Waltzinger, C. Benoist, and D. Mathis. 2001. How much TCR does a T cell need? *Immunity*. 15:71-82.

Langkamp-Henken, B., B.S. Bender, E.M. Gardner, K.A. Herrlinger-Garcia, M.J. Kelley, D.M. Murasko, J.P. Schaller, J.K. Stechmiller, D.J. Thomas, and S.M. Wood. 2004. Nutritional formula enhanced immune function and reduced days of symptoms of upper respiratory tract infection in seniors. *J Am Geriatr Soc*. 52:3-12.

Lee, K.A., R.A. Roth, and J.J. LaPres. 2007. Hypoxia, drug therapy and toxicity. *Pharmacol Ther*. 113:229-46.

Lee, S.H., N. Fujita, T. Mashima, and T. Tsuruo. 1996. Interleukin-7 inhibits apoptosis of mouse malignant T-lymphoma cells by both suppressing the CPP32-like protease activation and inducing the Bcl-2 expression. *Oncogene*. 13:2131-9.

Li, C.R., and L.J. Berg. 2005. Itk is not essential for CD28 signaling in naive T cells. *J Immunol*. 174:4475-9.

Li, W.Q., Q. Jiang, A.R. Khaled, J.R. Keller, and S.K. Durum. 2004. Interleukin-7 inactivates the pro-apoptotic protein Bad promoting T cell survival. *J Biol Chem*. 279:29160-6.

Liao, X.C., S. Fournier, N. Killeen, A. Weiss, J.P. Allison, and D.R. Littman. 1997. Itk negatively regulates induction of T cell proliferation by CD28 costimulation. *J Exp Med*. 186:221-8.

Liao, X.C., and D.R. Littman. 1995. Altered T cell receptor signaling and disrupted T cell development in mice lacking Itk. *Immunity*. 3:757-69.

Link, A., T.K. Vogt, S. Favre, M.R. Britschgi, H. Acha-Orbea, B. Hinz, J.G. Cyster, and S.A. Luther. 2007. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat Immunol*. 8:1255-65.

Lucas, P.J., I. Negishi, K. Nakayama, L.E. Fields, and D.Y. Loh. 1995. Naive CD28-deficient T cells can initiate but not sustain an in vitro antigen-specific immune response. *J Immunol*. 154:5757-68.

Macian, F. 2005. NFAT proteins: key regulators of T-cell development and function. *Nat Rev Immunol*. 5:472-84.

Maciver, N.J., S.R. Jacobs, H.L. Wieman, J.A. Wofford, J.L. Coloff, and J.C. Rathmell. 2008. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol*. 84:949-57.

Makni, K., F. Mnif, M. Boudawara, N. Hamza, N. Rekik, M. Abid, A. Rebai, F. Jarraya, C. Granier, and H. Ayadi. 2008. Association of glucose transporter 1 polymorphisms with type 2 diabetes in the Tunisian population. *Diabetes Metab Res Rev*. 24:544-8.

Maraskovsky, E., L.A. O'Reilly, M. Teepe, L.M. Corcoran, J.J. Peschon, and A. Strasser. 1997. Bcl-2 can rescue T lymphocyte development in interleukin-7 receptor-deficient mice but not in mutant rag-1-/- mice. *Cell*. 89:1011-9.

Maraskovsky, E., M. Teepe, P.J. Morrissey, S. Braddy, R.E. Miller, D.H. Lynch, and J.J. Peschon. 1996. Impaired survival and proliferation in IL-7 receptor-deficient peripheral T cells. *J Immunol*. 157:5315-23.

Market, E., and F.N. Papavasiliou. 2003. V(D)J recombination and the evolution of the adaptive immune system. *PLoS Biol*. 1:E16.

Martin, R., and H.F. McFarland. 1995. Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. *Crit Rev Clin Lab Sci*. 32:121-82.

Maurer, U., C. Charvet, A. Wagman, E. Dejardin, and G. D.R. 2006. Glycogen Synthase Kinase-3 Regulates Mitochondrial Outer Membrane Permeabilization and Apoptosis by Destabilization of MCL-1. *Mol Cell*. 21:749-760.

Mazzucchelli, R., and S.K. Durum. 2007. Interleukin-7 receptor expression: intelligent design. *Nat Rev Immunol*. 7:144-54.

Mazzucchelli, R., J.A. Hixon, R. Spolski, X. Chen, W.Q. Li, V.L. Hall, J. Willette-Brown, A.A. Hurwitz, W.J. Leonard, and S.K. Durum. 2008. Development of regulatory T cells requires IL-7Ralpha stimulation by IL-7 or TSLP. *Blood*. 112:3283-92.

McCune, J.M., M.B. Hanley, D. Cesar, R. Halvorsen, R. Hoh, D. Schmidt, E. Wieder, S. Deeks, S. Siler, R. Neese, and M. Hellerstein. 2000. Factors influencing T-cell turnover in HIV-1-seropositive patients. *J Clin Invest*. 105:R1-8.

McGowan, K.M., S.D. Long, and P.H. Pekala. 1995. Glucose transporter gene expression: regulation of transcription and mRNA stability. *Pharmacol Ther*. 66:465-505.

Michel, F., and O. Acuto. 2002. CD28 costimulation: a source of Vav-1 for TCR signaling with the help of SLP-76? *Sci STKE*. 2002:PE35.

Michel, F., G. Attal-Bonnefoy, G. Mangino, S. Mise-Omata, and O. Acuto. 2001. CD28 as a molecular amplifier extending TCR ligation and signaling capabilities. *Immunity*. 15:935-45.

Michel, F., L. Grimaud, L. Tuosto, and O. Acuto. 1998. Fyn and ZAP-70 are required for Vav phosphorylation in T cells stimulated by antigen-presenting cells. *J Biol Chem*. 273:31932-8.

Michel, F., G. Mangino, G. Attal-Bonnefoy, L. Tuosto, A. Alcover, A. Roumier, D. Olive, and O. Acuto. 2000. CD28 utilizes Vav-1 to enhance TCR-proximal signaling and NF-AT activation. *J Immunol*. 165:3820-9.

Michie, C.A., A. McLean, C. Alcock, and P.C. Beverley. 1992. Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature*. 360:264-5.

Moley, K.H., M.M. Chi, and M.M. Mueckler. 1998. Maternal hyperglycemia alters glucose transport and utilization in mouse preimplantation embryos. *Am J Physiol*. 275:E38-47.

Mueckler, M., C. Caruso, S.A. Baldwin, M. Panico, I. Blench, H.R. Morris, W.J. Allard, G.E. Lienhard, and H.F. Lodish. 1985. Sequence and structure of a human glucose transporter. *Science*. 229:941-5.

Mueckler, M., R.C. Hresko, and M. Sato. 1997. Structure, function and biosynthesis of GLUT1. *Biochem Soc Trans*. 25:951-4.

Murakami, T., T. Nishiyama, T. Shirotani, Y. Shinohara, M. Kan, K. Ishii, F. Kanai, S. Nakazuru, and Y. Ebina. 1992. Identification of two enhancer elements in the gene encoding the type 1 glucose transporter from the mouse which are responsive to serum, growth factor, and oncogenes. *J Biol Chem*. 267:9300-6.

Myung, P.S., G.S. Derimanov, M.S. Jordan, J.A. Punt, Q.H. Liu, B.A. Judd, E.E. Meyers, C.D. Sigmund, B.D. Freedman, and G.A. Koretzky. 2001. Differential requirement for SLP-76 domains in T cell development and function. *Immunity*. 15:1011-26.

Nakajima, H., and W.J. Leonard. 1999. Role of Bcl-2 in alpha beta T cell development in mice deficient in the common cytokine receptor gamma-chain: the requirement for Bcl-2 differs depending on the TCR/MHC affinity. *J Immunol*. 162:782-90.

Nakayama, K., K. Nakayama, L.B. Dustin, and D.Y. Loh. 1995. T-B cell interaction inhibits spontaneous apoptosis of mature lymphocytes in Bcl-2-deficient mice. *J Exp Med*. 182:1101-9.

Namen, A.E., S. Lupton, K. Hjerrild, J. Wignall, D.Y. Mochizuki, A. Schmierer, B. Mosley, C.J. March, D. Urdal, and S. Gillis. 1988. Stimulation of B-cell progenitors by cloned murine interleukin-7. *Nature*. 333:571-3.

Nikoulina, S.E., T.P. Ciaraldi, S. Mudaliar, L. Carter, K. Johnson, and R.R. Henry. 2002. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. *Diabetes*. 51:2190-8.

Noel, P.J., L.H. Boise, J.M. Green, and C.B. Thompson. 1996. CD28 costimulation prevents cell death during primary T cell activation. *J Immunol*. 157:636-42.

Nunes, J.A., Y. Collette, A. Truneh, D. Olive, and D.A. Cantrell. 1994. The role of p21ras in CD28 signal transduction: triggering of CD28 with antibodies, but not the ligand B7-1, activates p21ras. *J Exp Med*. 180:1067-76.

Onetti, R., J. Baulida, and A. Bassols. 1997. Increased glucose transport in ras-transformed fibroblasts: a possible role for N-glycosylation of GLUT1. *FEBS Lett*. 407:267-70.

Opferman, J.T., A. Letai, C. Beard, M.D. Sorcinelli, C.C. Ong, and S.J. Korsmeyer. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature*. 426:671-6.

Osborne, L.C., S. Dhanji, J.W. Snow, J.J. Priatel, M.C. Ma, M.J. Miners, H.S. Teh, M.A. Goldsmith, and N. Abraham. 2007. Impaired CD8 T cell memory and CD4 T cell primary responses in IL-7R alpha mutant mice. *J Exp Med*. 204:619-31.

Pages, F., M. Ragueneau, R. Rottapel, A. Truneh, J. Nunes, J. Imbert, and D. Olive. 1994. Binding of phosphatidylinositol-3-OH kinase to CD28 is required for T-cell signalling. *Nature*. 369:327-9.

Pallard, C., A.P. Stegmann, T. van Kleffens, F. Smart, A. Venkitaraman, and H. Spits. 1999. Distinct roles of the phosphatidylinositol 3-kinase and STAT5 pathways in IL-7-mediated development of human thymocyte precursors. *Immunity*. 10:525-35.

Park, C., S. Li, E. Cha, and C. Schindler. 2000. Immune response in Stat2 knockout mice. *Immunity*. 13:795-804.

Park, J.H., Q. Yu, B. Erman, J.S. Appelbaum, D. Montoya-Durango, H.L. Grimes, and A. Singer. 2004. Suppression of IL7Ralpha transcription by IL-7 and other prosurvival cytokines: a novel mechanism for maximizing IL-7-dependent T cell survival. *Immunity*. 21:289-302.

Pellegrini, M., P. Bouillet, M. Robati, G.T. Belz, G.M. Davey, and A. Strasser. 2004. Loss of Bim increases T cell production and function in interleukin 7 receptor-deficient mice. *J Exp Med*. 200:1189-95.

Pellegrini, M., T. Calzascia, A.R. Elford, A. Shahinian, A.E. Lin, D. Dissanayake, S. Dhanji, L.T. Nguyen, M.A. Gronski, M. Morre, B. Assouline, K. Lahl, T. Sparwasser, P.S. Ohashi, and T.W. Mak. 2009. Adjuvant IL-7 antagonizes multiple cellular and molecular inhibitory networks to enhance immunotherapies. *Nat Med*. 15:528-36.

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.

Piccio, L., J.L. Stark, and A.H. Cross. 2008. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J Leukoc Biol*. 84:940-8.

Piper, R.C., L.J. Hess, and D.E. James. 1991. Differential sorting of two glucose transporters expressed in insulin-sensitive cells. *Am J Physiol*. 260:C570-80.

Plas, D.R., J.C. Rathmell, and C.B. Thompson. 2002. Homeostatic control of lymphocyte survival: potential origins and implications. *Nat Immunol*. 3:515-21.

Plas, D.R., S. Talapatra, A.L. Edinger, J.C. Rathmell, and C.B. Thompson. 2001. Akt and Bcl-xL promote growth factor-independent survival through distinct effects on mitochondrial physiology. *J Biol Chem*. 276:12041-8.

Plas, D.R., and C.B. Thompson. 2005. Akt-dependent transformation: there is more to growth than just surviving. *Oncogene*. 24:7435-42.

Poetschke, H.L., D.B. Klug, S.N. Perkins, T.T. Wang, E.R. Richie, and S.D. Hursting. 2000. Effects of calorie restriction on thymocyte growth, death and maturation. *Carcinogenesis*. 21:1959-64.

Polic, B., D. Kunkel, A. Scheffold, and K. Rajewsky. 2001. How alpha beta T cells deal with induced TCR alpha ablation. *Proc Natl Acad Sci U S A*. 98:8744-9.

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.

Pui, J.C., D. Allman, L. Xu, S. DeRocco, F.G. Karnell, S. Bakkour, J.Y. Lee, T. Kadesch, R.R. Hardy, J.C. Aster, and W.S. Pear. 1999. Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity*. 11:299-308.

Purohit, S.J., R.P. Stephan, H.G. Kim, B.R. Herrin, L. Gartland, and C.A. Klug. 2003. Determination of lymphoid cell fate is dependent on the expression status of the IL-7 receptor. *Embo J*. 22:5511-21.

Raff, M.C. 1992. Social controls on cell survival and cell death. *Nature*. 356:397-400.

Ranger, A.M., J. Zha, H. Harada, S.R. Datta, N.N. Danial, A.P. Gilmore, J.L. Kutok, M.M. Le Beau, M.E. Greenberg, and S.J. Korsmeyer. 2003. Bad-deficient mice develop diffuse large B cell lymphoma. *Proc Natl Acad Sci U S A*. 100:9324-9.

Rathmell, J.C., R.L. Elstrom, R.M. Cinalli, and C.B. Thompson. 2003a. Activated Akt promotes increased resting T cell size, CD28-independent T cell growth, and development of autoimmunity and lymphoma. *Eur J Immunol*. 33:2223-32.

Rathmell, J.C., E.A. Farkash, W. Gao, and C.B. Thompson. 2001. IL-7 enhances the survival and maintains the size of naive T cells. *J Immunol*. 167:6869-76.

Rathmell, J.C., C.J. Fox, D.R. Plas, P.S. Hammerman, R.M. Cinalli, and C.B. Thompson. 2003b. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. *Mol Cell Biol*. 23:7315-28.

Rathmell, J.C., T. Lindsten, W.X. Zong, R.M. Cinalli, and C.B. Thompson. 2002. Deficiency in Bak and Bax perturbs thymic selection and lymphoid homeostasis. *Nat Immunol*. 3:932-9.

Rathmell, J.C., M.G. Vander Heiden, M.H. Harris, K.A. Frauwirth, and C.B. Thompson. 2000. In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. *Mol Cell*. 6:683-92.

Reed, B.C., C. Cefalu, B.H. Bellaire, J.A. Cardelli, T. Louis, J. Salamon, M.A. Bloecher, and R.C. Bunn. 2005. GLUT1CBP(TIP2/GIPC1) interactions with GLUT1 and myosin VI: evidence supporting an adapter function for GLUT1CBP. *Mol Biol Cell*. 16:4183-201.

Reynolds, L.F., L.A. Smyth, T. Norton, N. Freshney, J. Downward, D. Kioussis, and V.L. Tybulewicz. 2002. Vav1 transduces T cell receptor signals to the activation of phospholipase C-gamma1 via phosphoinositide 3-kinase-dependent and -independent pathways. *J Exp Med*. 195:1103-14.

Rich, B.E., J. Campos-Torres, R.I. Tepper, R.W. Moreadith, and P. Leder. 1993. Cutaneous lymphoproliferation and lymphomas in interleukin 7 transgenic mice. *J Exp Med*. 177:305-16.

Riley, J.L., and C.H. June. 2005. The CD28 family: a T-cell rheostat for therapeutic control of T-cell activation. *Blood*. 105:13-21.

Riley, J.L., M. Mao, S. Kobayashi, M. Biery, J. Burchard, G. Cavet, B.P. Gregson, C.H. June, and P.S. Linsley. 2002. Modulation of TCR-induced transcriptional profiles by ligation of CD28, ICOS, and CTLA-4 receptors. *Proc Natl Acad Sci U S A*. 99:11790-5.

Rinkenberger, J.L., S. Horning, B. Klocke, K. Roth, and S.J. Korsmeyer. 2000. Mcl-1 deficiency results in peri-implantation embryonic lethality. *Genes Dev*. 14:23-7.

Robey, E., and B.J. Fowlkes. 1994. Selective events in T cell development. *Annu Rev Immunol*. 12:675-705.

Rodig, S.J., M.A. Meraz, J.M. White, P.A. Lampe, J.K. Riley, C.D. Arthur, K.L. King, K.C. Sheehan, L. Yin, D. Pennica, E.M. Johnson, Jr., and R.D. Schreiber. 1998.

Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell*. 93:373-83.

Roos, D., and J.A. Loos. 1973. Changes in the carbohydrate metabolism of mitogenically stimulated human peripheral lymphocytes. II. Relative importance of glycolysis and oxidative phosphorylation on phytohaemagglutinin stimulation. *Exp Cell Res*. 77:127-35.

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.

Rudd, C.E., A. Taylor, and H. Schneider. 2009. CD28 and CTLA-4 coreceptor expression and signal transduction. *Immunol Rev*. 229:12-26.

Ruzankina, Y., C. Pinzon-Guzman, A. Asare, T. Ong, L. Pontano, G. Cotsarelis, V.P. Zediak, M. Velez, A. Bhandoola, and E.J. Brown. 2007. Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. *Cell Stem Cell*. 1:113-26.

Sagone, A.L., Jr., A.F. LoBuglio, and S.P. Balcerzak. 1974. Alterations in hexose monophosphate shunt during lymphoblastic transformation. *Cell Immunol*. 14:443-52.

Samaridis, J., G. Casorati, A. Traunecker, A. Iglesias, J.C. Gutierrez, U. Muller, and R. Palacios. 1991. Development of lymphocytes in interleukin 7-transgenic mice. *Eur J Immunol*. 21:453-60.

Sanchez-Feutrie, M., F. Vinals, M. Palacin, and A. Zorzano. 2003. Identification of a novel proliferation-dependent C-rich element that mediates inhibition of the rat GLUT1 promoter. *Gene*. 322:47-55.

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

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

Schneider, H., and C.E. Rudd. 2008. CD28 and Grb-2, relative to Gads or Grap, preferentially co-operate with Vav1 in the activation of NFAT/AP-1 transcription. *Biochem Biophys Res Commun*. 369:616-21.

Scollay, R. 1991. T-cell subset relationships in thymocyte development. *Curr Opin Immunol*. 3:204-9.

Seddon, B., and R. Zamoyska. 2002. TCR signals mediated by Src family kinases are essential for the survival of naive T cells. *J Immunol*. 169:2997-3005.

Semenza, G.L., D. Artemov, A. Bedi, Z. Bhujwalla, K. Chiles, D. Feldser, E. Laughner, R. Ravi, J. Simons, P. Taghavi, and H. Zhong. 2001. 'The metabolism of tumours': 70 years later. *Novartis Found Symp*. 240:251-60; discussion 260-4.

Sentman, C.L., J.R. Shutter, D. Hockenbery, O. Kanagawa, and S.J. Korsmeyer. 1991. bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell*. 67:879-88.

Shen, S., M. Zhu, J. Lau, M. Chuck, and W. Zhang. 2009. The essential role of LAT in thymocyte development during transition from the double-positive to single-positive stage. *J Immunol*. 182:5596-604.

Smith-Garvin, J.E., G.A. Koretzky, and M.S. Jordan. 2009. T cell activation. *Annu Rev Immunol*. 27:591-619.

Starr, T.K., S.C. Jameson, and K.A. Hogquist. 2003. Positive and negative selection of T cells. *Annu Rev Immunol*. 21:139-76.

Strasser, A., A.W. Harris, and S. Cory. 1991. bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. *Cell*. 67:889-99.

Strasser, A., L. O'Connor, D.C. Huang, L.A. O'Reilly, M.L. Stanley, M.L. Bath, J.M. Adams, S. Cory, and A.W. Harris. 1996. Lessons from bcl-2 transgenic mice for immunology, cancer biology and cell death research. *Behring Inst Mitt*:101-17.

Su, B., E. Jacinto, M. Hibi, T. Kallunki, M. Karin, and Y. Ben-Neriah. 1994. JNK is involved in signal integration during costimulation of T lymphocytes. *Cell*. 77:727-36.

Surh, C.D., O. Boyman, J.F. Purton, and J. Sprent. 2006. Homeostasis of memory T cells. *Immunol Rev*. 211:154-63.

Surh, C.D., and J. Sprent. 2008. Homeostasis of naive and memory T cells. *Immunity*. 29:848-62.

Swainson, L., S. Kinet, N. Manel, J.L. Battini, M. Sitbon, and N. Taylor. 2005. Glucose transporter 1 expression identifies a population of cycling CD4+ CD8+ human

thymocytes with high CXCR4-induced chemotaxis. *Proc Natl Acad Sci U S A*. 102:12867-72.

Sweeney, E.B., F.M. Foss, J.R. Murphy, and J.C. vanderSpek. 1998. Interleukin 7 (IL-7) receptor-specific cell killing by DAB389 IL-7: a novel agent for the elimination of IL-7 receptor positive cells. *Bioconjug Chem*. 9:201-7.

Takeda, K., T. Kaisho, N. Yoshida, J. Takeda, T. Kishimoto, and S. Akira. 1998. Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. *J Immunol*. 161:4652-60.

Takeda, S., H.R. Rodewald, H. Arakawa, H. Bluethmann, and T. Shimizu. 1996. MHC class II molecules are not required for survival of newly generated CD4+ T cells, but affect their long-term life span. *Immunity*. 5:217-28.

Tamas, P., S.A. Hawley, R.G. Clarke, K.J. Mustard, K. Green, D.G. Hardie, and D.A. Cantrell. 2006. Regulation of the energy sensor AMP-activated protein kinase by antigen receptor and Ca2+ in T lymphocytes. *J Exp Med*. 203:1665-70.

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.

Tan, J.T., B. Ernst, W.C. Kieper, E. LeRoy, J. Sprent, and C.D. Surh. 2002. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. *J Exp Med*. 195:1523-32.

Tanchot, C., F.A. Lemonnier, B. Perarnau, A.A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science*. 276:2057-62.

Teglund, S., C. McKay, E. Schuetz, J.M. van Deursen, D. Stravopodis, D. Wang, M. Brown, S. Bodner, G. Grosveld, and J.N. Ihle. 1998. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell*. 93:841-50.

Tollefsbol, T.O., and H.J. Cohen. 1985. Culture kinetics of glycolytic enzyme induction, glucose utilization, and thymidine incorporation of extended-exposure phytohemagglutinin-stimulated human lymphocytes. *J Cell Physiol*. 122:98-104.

Tremblay, F., A. Gagnon, A. Veilleux, A. Sorisky, and A. Marette. 2005. Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to

Akt and glucose transport in 3T3-L1 and human adipocytes. *Endocrinology*. 146:1328-37.

Tuosto, L., F. Michel, and O. Acuto. 1996. p95vav associates with tyrosine-phosphorylated SLP-76 in antigen-stimulated T cells. *J Exp Med*. 184:1161-6.

Turner, M., and D.D. Billadeau. 2002. VAV proteins as signal integrators for multi-subunit immune-recognition receptors. *Nat Rev Immunol*. 2:476-86.

Ullrich, O., S. Reinsch, S. Urbe, M. Zerial, and R.G. Parton. 1996. Rab11 regulates recycling through the pericentriolar recycling endosome. *J Cell Biol*. 135:913-24.

Uribe, L., and K.I. Weinberg. 1998. X-linked SCID and other defects of cytokine pathways. *Semin Hematol*. 35:299-309.

Vander Heiden, M.G., L.C. Cantley, and C.B. Thompson. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 324:1029-33.

Vander Heiden, M.G., D.R. Plas, J.C. Rathmell, C.J. Fox, M.H. Harris, and C.B. Thompson. 2001. Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol*. 21:5899-912.

Veis, D.J., C.M. Sorenson, J.R. Shutter, and S.J. Korsmeyer. 1993. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell*. 75:229-40.

von Boehmer, H., and H.J. Fehling. 1997. Structure and function of the pre-T cell receptor. *Annu Rev Immunol*. 15:433-52.

von Freeden-Jeffry, U., N. Solvason, M. Howard, and R. Murray. 1997. The earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression. *Immunity*. 7:147-54.

von Freeden-Jeffry, U., P. Vieira, L.A. Lucian, T. McNeil, S.E. 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.

Vranjkovic, A., A.M. Crawley, K. Gee, A. Kumar, and J.B. 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.

Warburg, O. 1956. On the origin of cancer cells. *Science*. 123:309-14.

Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*. 356:314-7.

Weant, A.E., R.D. Michalek, I.U. Khan, B.C. Holbrook, M.C. Willingham, and J.M. Grayson. 2008. Apoptosis regulators Bim and Fas function concurrently to control autoimmunity and CD8+ T cell contraction. *Immunity*. 28:218-30.

Webb, S., C. Morris, and J. Sprent. 1990. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell*. 63:1249-56.

Welsh, G.I., I. Hers, D.C. Berwick, G. Dell, M. Wherlock, R. Birkin, S. Leney, and J.M. Tavare. 2005. Role of protein kinase B in insulin-regulated glucose uptake. *Biochem Soc Trans*. 33:346-9.

Wieman, H.L., S.R. Horn, S.R. Jacobs, B.J. Altman, S. Kornbluth, and J.C. Rathmell. 2009. An essential role for the Glut1 PDZ-binding motif in growth factor regulation of Glut1 degradation and trafficking. *Biochem J*. 418:345-67.

Wieman, H.L., J.A. Wofford, and J.C. Rathmell. 2007. Cytokine Stimulation Promotes Glucose Uptake via PI3K/Akt Regulation of Glut1 Activity and Trafficking. *Mol Biol Cell*. 18:1437-1446.

Wiles, M.V., P. Ruiz, and B.A. Imhof. 1992. Interleukin-7 expression during mouse thymus development. *Eur J Immunol*. 22:1037-42.

Willis, S.N., L. Chen, G. Dewson, A. Wei, E. Naik, J.I. Fletcher, J.M. Adams, and D.C. Huang. 2005. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. *Genes Dev*. 19:1294-305.

Wofford, J.A., H.L. Wieman, S.R. Jacobs, Y. Zhao, and J.C. Rathmell. 2008. IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. *Blood*. 111:2101-11.

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

Wright, E.M., and E. Turk. 2004. The sodium/glucose cotransport family SLC5. *Pflugers Arch*. 447:510-8.

Wuest, T.Y., J. Willette-Brown, S.K. Durum, and A.A. Hurwitz. 2008. The influence of IL-2 family cytokines on activation and function of naturally occurring regulatory T cells. *J Leukoc Biol*. 84:973-80.

Xue, H.H., P.E. Kovanen, C.A. Pise-Masison, M. Berg, M.F. Radovich, J.N. Brady, and W.J. Leonard. 2002. IL-2 negatively regulates IL-7 receptor alpha chain expression in activated T lymphocytes. *Proc Natl Acad Sci U S A*. 99:13759-64.

Yang, T.T., H.Y. Suk, X. Yang, O. Olabisi, R.Y. Yu, J. Durand, L.A. Jelicks, J.Y. Kim, P.E. Scherer, Y. Wang, Y. Feng, L. Rossetti, I.A. Graef, G.R. Crabtree, and C.W. Chow. 2006. Role of transcription factor NFAT in glucose and insulin homeostasis. *Mol Cell Biol*. 26:7372-87.

Yao, Z., Y. Cui, W.T. Watford, J.H. Bream, K. Yamaoka, B.D. Hissong, D. Li, S.K. Durum, Q. Jiang, A. Bhandoola, L. Hennighausen, and J.J. O'Shea. 2006. Stat5a/b are essential for normal lymphoid development and differentiation. *Proc Natl Acad Sci U S A*. 103:1000-5.

Yu, Q., B. Erman, A. Bhandoola, S.O. Sharrow, and A. Singer. 2003. In vitro evidence that cytokine receptor signals are required for differentiation of double positive thymocytes into functionally mature CD8+ T cells. *J Exp Med*. 197:475-87.

Zhang, W., C.L. Sommers, D.N. Burshtyn, C.C. Stebbins, J.B. DeJarnette, R.P. Trible, A. Grinberg, H.C. Tsay, H.M. Jacobs, C.M. Kessler, E.O. Long, P.E. Love, and L.E. Samelson. 1999. Essential role of LAT in T cell development. *Immunity*. 10:323-32.

Zhao, F.Q., D.R. Glimm, and J.J. Kennelly. 1993. Distribution of mammalian facilitative glucose transporter messenger RNA in bovine tissues. *Int J Biochem*. 25:1897-903.

Zhao, F.Q., and A.F. Keating. 2007. Functional properties and genomics of glucose transporters. *Curr Genomics*. 8:113-28.

Zhao, Y., B.J. Altman, J.L. Coloff, C.E. Herman, S.R. Jacobs, H.L. Wieman, J.A. Wofford, L.N. Dimascio, O. Ilkayeva, A. Kelekar, T. Reya, and J.C. Rathmell. 2007. Glycogen Synthase Kinase 3{alpha} and 3{beta} Mediate a Glucose-Sensitive Antiapoptotic Signaling Pathway To Stabilize Mcl-1. *Mol Cell Biol*. 27:4328-39.

Zhao, Y., J.L. Coloff, E.C. Ferguson, S.R. Jacobs, K. Cui, and J.C. Rathmell. 2008. Glucose metabolism attenuates p53 and Puma-dependent cell death upon growth factor deprivation. *J Biol Chem*. 283:36344-53.

Zhou, P., N.B. Levy, H. Xie, L. Qian, C.Y. Lee, R.D. Gascoyne, and R.W. Craig. 2001. MCL1 transgenic mice exhibit a high incidence of B-cell lymphoma manifested as a spectrum of histologic subtypes. *Blood*. 97:3902-9.

Zhou, P., L. Qian, C.K. Bieszczad, R. Noelle, M. Binder, N.B. Levy, and R.W. Craig. 1998. Mcl-1 in transgenic mice promotes survival in a spectrum of hematopoietic cell types and immortalization in the myeloid lineage. *Blood*. 92:3226-39.

Biography

Sarah Ruth Jacobs

Born to William and Karen Jacobs on July 27, 1982 in Beaumont, Texas.

Married to Nicholas Holifield on May 31, 2008 in Durham, North Carolina.

Education

Ph.D. Molecular Cancer Biology
Duke University, Durham, North Carolina, June 2009

B.S. Molecular and Cellular Biology, with Highest Honors
University of Texas at Austin, Austin, Texas, May 2004

Publications

Jacobs, S.R., R.D. Michalek, and J.C. Rathmell. (2009) Interleukin 7 is essential to maintain T cell glycolysis in vivo. *In preparation.*

Wieman, H.L., S.R. Horn**, S.R. Jacobs**, B.J. Altman, S. Kornbluth and J.C. Rathmell. (2009) An essential role for the Glut1 PDZ-binding motif in growth factor regulation of Glut1 degradation and trafficking. *Biochemical Journal*, 418:345-357.

Zhao, Y., J.L. Coloff, E.C. Ferguson, **S.R. Jacobs**, K. Cui, and J.C. Rathmell. (2008) Glucose metabolism attenuates p53 and Puma-dependent cell death upon growth factor deprivation. *Journal of Biological Chemistry*, 283:36344-36353.

Zhao, Y., H.L. Wieman, **S.R. Jacobs**, and J.C. Rathmell. (2008) Mechanisms and methods in glucose metabolism and cell death. *Methods in Enzymology*, 442:439-457.

MacIver, N.J., **S.R. Jacobs**, H.L. Wieman, J.A. Wofford, J.L. Coloff, and J.C. Rathmell. (2008) Glucose metabolism in lymphocytes is a regulated process with significant effects on function and survival. *Journal of Leukocyte Biology*, 84(4):949-957.

Jacobs, S.R., C.E. Herman, N.J. MacIver, J.A. Wofford, H.L. Wieman, J.J. Hammen, and J.C. Rathmell. (2008) Glucose Uptake is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *Journal of Immunology,* 180: 4476-4486.

Wofford, J.A., H.L. Wieman, **S.R. Jacobs**, Y. Zhao, and J.C. Rathmell. (2008) IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T cell survival. *Blood*. 111:2101-2111.

Zhao, Y., B.J. Altman, J.L. Coloff, C.E. Herman, **S.R. Jacobs**, H.L. Wieman, J.A. Wofford, L.N. Dimascio, O. Ilkayeva, A. Kelekar, T. Reya, and J.C. Rathmell. (2007) GSK-3a/b Mediate a Glucose-Sensitive Anti-Apoptotic Signaling Pathway to Stabilize Mcl-1. *Molecular and Cellular Biology* 12: 4328-4339.

Gerardo, N.M., **S.R. Jacobs**, C.R. Currie, U.G. Mueller, (2006) Ancient host-pathogen associations maintained by specificity of chemotaxis and antibiosis. *Public Library of Science* 4: 235.

Jacobs, S.R. and J.C. Rathmell, (2006) Lymphocyte Selection by Starvation: Glucose Metabolism and Cell Death. *Trends in Immunology* 27: 4-7.