METABOLIC REGULATION OF THE ANTI-HELMINTH CD4$^+$ T CELL RESPONSE

by

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Committee Page

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Abstract

Cellular metabolism is intricately linked to the activation and effector function of $CD4^+$ T cells. Activation triggers an increase in glycolysis, which is thought to fuel the necessary biosynthetic demands of growth and proliferation. Recent work suggests that glycolytic activity further coordinates distinct functions in different T helper subsets. Thus far, however, little has been elucidated about the regulation of metabolism in Th2 cells.

Using *in vitro* cultures and *in vivo* infection models, I compared glycolysis in Th1 and Th2 cells generated from cytokine-reporter mice. This revealed that Th2 cells from infected mice maintain a low glycolytic rate that more closely resembles that of naïve T cells. I hypothesized Th2 cells may be metabolically suppressed by helminths during infection, and therefore tested the capability of purified helminth products to alter glycolysis of *in vitro* polarized Th2 cells. Cytokine production was inhibited, but an effect on glycolysis could not be consistently demonstrated. However, signalling through the metabolic regulator, mTOR, was reduced in T cells from helminth infected mice compared to controls, supporting the possibility of metabolic modulation *in vivo*.

I questioned whether Th2 cell glycolysis was regulated after exiting the lymph node. By assessing Th2 cell metabolism from the effector site, I discovered that fatty acid metabolism, and not glycolysis, was upregulated in the peripheral tissue. Fatty acid uptake was enhanced in cells expressing the IL-33 receptor, ST2. These

cells highly co-expressed programmed death protein 1 (PD-1), a known regulator of T cell metabolism. Hence, I predicted that PD-1 signalling promotes ST2 expression in the tissue. Accordingly, *ex vivo* stimulation of sorted Th2 cells through PD-1 increased ST2 expression. From these data I propose that PD-1 signalling overcomes a metabolic checkpoint to permit alarmin-responsiveness and tissue-localized cytokine production.

Overall, these findings illustrate Th2 metabolism is dynamic *in vivo*, and that fatty acid metabolism is the predominant pathway regulated during effector differentiation. Importantly, these results argue that immunometabolism data from *in vitro* systems may not be applicable *in vivo*. Continuing to understand *in vivo* Th2 cell metabolism could be pertinent to the future design of anti-helminth treatments, which are urgently needed.

Lay Summary

T cells are a type of white blood cell that provide instructions to other immune cells in order to coordinate protection against infection. During infection, T cells change their metabolism, or the collective chemical reactions used to produce cellular energy and molecules needed for growth and division. How T cell metabolism is altered may determine the signals used by T cells to instruct protective immunity against different infectious organisms. This research aims to understand how T cell metabolism is different when fighting large parasitic worms, or helminths, compared to microscopic organisms such as bacteria or viruses. The main finding is that, during helminth infection, T cells preferentially rely on molecules from fats, whereas T cells from other infections predominantly use sugars. This work highlights important considerations needed for the design of immune-based therapies, such as vaccination against helminth infection, which impacts 2 billion people world-wide.

Preface

This dissertation presents original unpublished research. Work was divided between the University of British Columbia, Canada, and the University of Glasgow, United Kingdom. I designed and performed all experiments under the supervision of Dr. Georgia Perona-Wright, with help from the following colleagues:

- The Seahorse protocol was optimized and provided by Dan Wu of Prof. Megan Levings' group at BC Children's Hospital
- i.v. injections of 2NBDG for Fig. 3.6 were done by Dr. Sally Lee-Sayer
- Holly Webster and Amy Shergold, a PhD student and research assistant in the lab, helped set-up and analyse select *in vitro* cultures in Glasgow
- The HDM protocol for Fig. 5.3 was designed and carried out with the help of Dr. Stephan Loeser, a post-doctoral researcher with Prof. Rick Maizels.

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2DG	2-Deoxy-D-Glucose
AAM	Alternatively Activated Macrophages
ACC1	Acetyl-CoA Carboxylase 1 (*ACACA* gene*)*
ACLY	ATP Citrate Lyase
AMPK	AMP Activated Protein Kinase
AP-1	Activator Protein 1
APC	Antigen Presenting Cell
ATP	Adenosine Triphosphate
BCL-6	B-Cell Lymphoma 6
CCL	C-C motif Chemokine Ligand
CCR	C-C motif Chemokine Receptor
CD	Cluster of Differentiation
CPT1A	Carnitine Palmitoyltransferase 1A
CTLA4	Cytotoxic T-Lymphocyte Protein 4
CXCL	C-X-C motif Ligand
CXCR	C-X-C motif Receptor
DC	Dendritic Cell
DN	Double-Negative
DP	Double-Positive
EAE	Experimental Autoimmune Encephalomyelitis
ECAR	Extracellular Acidification Rate
ENO1	Enolase 1

ETC	Electron Transport Chain
FA	Fatty Acids
FABP	Fatty Acid Binding Protein
FACS	Fluorescence Activated Cell Sorting
$FADH_2$	Flavin Adenine Dinucleotide (Hydroquinone form)
FAO	Fatty Acid Oxidation
FAS	Fatty Acid Synthesis
FASN	Fatty Acid Synthase
FATP	Fatty Acid Transporter
Foxp3	Forkhead Box P3
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GATA3	GATA Binding Protein 3
GC	Germinal Centre
GFP/YFP	Green/Yellow Fluorescent Protein
GLUT	Glucose Transporter
GVHD	Graft Versus Host Disease
HDM	House Dust-Mite
HES	*H. polygyrus* Excretions/Secretions
HIF1α	Hypoxia-Inducible Factor-1α
HK2	Hexokinase 2
IFNγ	Interferon γ
Ig(G,E)	Immunoglobulin (class G, class E)
IL-	Interleukin

IL-2Rα	IL-2 Receptor α
IL-4Rα	IL-4 Receptor α
ILC	Innate Lymphoid Cell
LCMV	Lymphocytic Choriomeningitis Virus
LDHA	Lactate Dehydrogenase A
MdLN	Mediastinal Lymph Node
MHC	Major Histocompatibility Complex
MHCII	MHC Class II
MLN	Mesenteric Lymph Node
mTOR	Mammalian Target of Rapamycin
mTORC1	mTOR Complex 1
mTORC2	mTOR Complex 2
NADH	Nicotinamide Adenine Dinucleotide
NFAT	Nuclear Factor of Activated T cells
NFκB	Nuclear Factor κ-light-chain-enhancer of activated B cells
OCR	Oxygen Consumption Rate
OXPHOS	Oxidative Phosphorylation
PD-1	Programmed Death Protein 1
PD-L1/2	Programmed Death-Ligand 1/2
PGC1α	PPARγ co-activator 1α
PI3K	Phosphoinositide 3-Kinase
PKM2	Pyruvate Kinase M2
PMA	Phorbol 12-myristate 13-acetate

PPAR	Peroxisome proliferator-activated receptors (α, β/δ, γ)
PPP	Pentose Phosphate Pathway
qPCR	Quantitative Polymerase Chain Reaction
RORγt	Retinoic Acid Receptor (RAR)-Related Orphan Receptor γ t
ROS	Reactive Oxygen Species
RPS29	Ribosomal Protein Subunit 29
SREBP	Sterol Regulatory Element-Binding Protein
ST2/IL1RL1	Serum Stimulation-2/IL-1 Receptor Like 1 (IL-33 receptor)
STAT	Signal Transducer and Activator of Transcription
TCA	Tricarboxylic Acid (cycle)
TCR	T Cell Receptor
Teff	Effector T cell
Tfam	Transcription factor A, mitochondrial
Tfh	T follicular helper cell
TGF-β	Transforming Growth Factor-β
TGM	TGF-β Mimic
Th	T helper
Treg	Regulatory T cell
TSLP	Thymic Stromal Lymphopoietin
UTR	Untranslated Region
VAL	Venom Allergen Like

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Chapter 1: Introduction

The immune system is an evolved network of cells and signals specialized to defend the host from infectious disease. It is divided into innate responders that act immediately and broadly against pathogens, or into adaptive responders that provide a second line of targeted and long-lasting protection against a particular infectious agent. $CD4^+$ T cells are central to the adaptive response. The fine tuning of T cell function is critical to maintaining host health while promoting pathogen clearance. A weak, unwanted or maladapted T cell response may mean chronic infection or immune-driven disease[1].

1.1 Generation and diversity of $CD4^+$ T cell responses

Activated $CD4^+$ T cells have the potential to acquire diverse phenotypes, each specialized for a certain scenario of infection[1]. T cell activation is instigated by recognition of a particular peptide by the T cell receptor (TCR), when presented on the major histocompatibility complex (MHC) by an antigen presenting cell (APC)[2]. $CD4^+$ T cells specifically recognize peptide presented by MHC class II (MHCII). TCR specificity is pre-determined during T cell development, but functional specificity, however, is a consequence of the milieu of signals encountered during and after activation[1,3]. An activated T cell is then defined by the cytokines, or soluble protein messengers, it secretes in order to orchestrate an immune response[1].

1.1.1 T cell development

T cells mature in the thymus from hematopoietic stem cell progenitors that enter from the bone marrow[3]. Thymic progenitors form a complete TCR complex with a paired α and β chain, that are progressively rearranged in order to recognize a particular peptide sequence when presented by MHC. Several selection checkpoints are employed to ensure that the TCR is functional, but does not react too strongly to self-peptides[4]. During positive selection, if the resulting TCR is unable to bind peptide-MHC (pMHC) with sufficient affinity, the cell is then programmed for apoptosis. This process also determines co-receptor expression of either CD4 or CD8 to become either classical α/β $CD4^+$ or a cytotoxic $CD8^+$ T cell, depending on whether the recognized peptide is displayed on MHC class I for CD8, or MHCII for CD4[3]. Through negative selection, T cells are similarly directed towards apoptosis following a high-affinity interaction with self-pMHC[3,4]. Those that survive positive and negative selection upregulate receptors permitting thymic egress, and entry into circulation towards secondary lymphoid organs such as peripheral lymph nodes[5].

1.1.2 Lymphocyte dynamics in the lymph node

Lymph nodes are densely packed, highly organized structures that provide hubs for lymphocytes to survey and encounter antigen as they circulate throughout the body. Naïve B cells and T cells form the largest constituency of trafficking cells within the lymph node, and enter via high endothelial venules, temporarily leaving circulation. Exit from the bloodstream is aided by expression of adhesive receptors

such as L-selection (CD62L) that bind high endothelial venules. Lymphocytes entering the lymph node then respectively transit to segregated T cell zones or B cell follicles accordingly, where they scan for their specific antigen[6].

Lymph node architecture is created and preserved by stromal cells that provide a scaffold for cell migration and enforce regional borders through the production of chemokine gradients[7]. The chemokines CCL19 and CCL21 attract naïve T and B lymphocytes expressing the receptor CCR7 into the lymph node. B cells are further drawn into the B cell follicle via expression of CXCR5, which ligates the chemokine CXCL13[6,7]. The areas immediately adjacent to the B cell follicle form an interface where T cells and B cells can form critical interactions during activation[8].

The timing of lymph node residence by naïve T cells (or B cells) of a given specificity must align with the delivery of their cognate antigen by professional APCs, specifically dendritic cells (DCs)[9]. Successful encounter of recognized peptide by naïve T lymphocytes triggers activation and functional differentiation. During an active immune response, the lymph node enlarges up to 10-times its homeostatic size to accommodate the enhanced recruitment and rapid division of cells[10]. Activated T cells at the T-B cell border provide signals to help B cell activation. Activated B cells go on to form germinal centres (GC) within the follicle, where B cells undergo differentiation and antibody class-switching[8]. The majority of activated T cells will then migrate out of the lymph node to the affected peripheral sites. If naïve cells fail to encounter the matching pMHC to their selected

TCR, they will similarly emigrate, but instead home to the next lymph node to repeat their antigen surveillance at a new site[6].

1.1.3 T cell activation

Activation of naïve T cells triggers transcriptional and metabolic changes in the cell that promote growth, proliferation, effector function and migration [2,11]. Optimal $CD4^+$ T cell activation requires multiple signals. DCs carrying processed antigen from peripheral sites prime naïve cells for activation by presenting peptide on MHCII, providing the first signal through TCR recognition[9]. Signalling via the CD28 co-stimulatory receptor on T cells provides a second essential signal when ligated by CD80 or CD86 expressed on DCs[12]. Downstream signalling of both receptors converges on a phosphorylation cascade to promote the nuclear translocation of transcription factors, such as NFκB, AP-1 and NFAT, to coordinate changes in gene expression needed for activation[11,12]. Isolated TCR signalling in the absence of CD28 during primary activation promotes a state of anergy, or unresponsiveness, whereas CD28 signalling on its own appears to have little impact on naïve T cells[11]. CD28 supports full T cell activation by amplifying TCR signals as well as engaging TCR-independent signalling pathways that facilitate changes in metabolism, cytoskeletal remodelling and transcriptional modifications[12]. Experimentally, during the primary immune response, activated T cells can be characterized by the loss of CCR7 and CD62L expression, and the up-regulation of CD69 and CD44. Mechanistically, these changes aid lymph node egress and promote entry into, and retention in, peripheral tissues.

1.1.4 Differentiation and effector function

Activated $CD4^+$ T cells are classified as several different subsets[1]. Subsets have divergent functions that are carried out by the expression of distinct sets of cytokines (Fig. 1.1). Though subsets are generally studied as distinct lineages, the current paradigm is now shifting towards an understanding that functional specification is not fixed but rather plastic in nature[13]. Despite this, immune responses are still labelled according to most prevalent subset detected, which ultimately determines the outcome of infection or disease.

T helper 1 cells

T helper (Th)1 cells orchestrate "type 1" immune responses required for the elimination of intracellular bacteria, viruses or protozoa, as well as the eradication of tumour cells[14]. Ultimately clearance depends on the death and disposal of the affected cells, dependent on $CD8^+$ cytotoxic T cell activation and the classical activation of macrophages. Th1 cells are defined by the transcription factor T-bet, and production of interferon (IFN)γ. Polarisation towards Th1 requires IFNγ itself, sourced from nearby innate cells, as well as IL-12 produced by mature DCs. The binding of IFNγ to its receptor signals through activated STAT1 (signal transducer and activator of transcription 1) to drive T-bet expression. T-bet directly binds to, and positively regulates the *ifng* gene locus, and enforces expression of the IL-12 receptor, which signals via STAT4. This creates a positive feedback loop in which IL-12 activation of STAT4 exaggerates IFNγ production by directly targeting its gene locus also.

T helper 17 cells

Th17 responses occur in reaction to fungal and some extracellular bacterial infections[15]. Th17 cells are also viewed as predominant drivers of several auto-immune diseases, such as multiple sclerosis, rheumatoid arthritis and psoriasis. Th17 cells characteristically secrete IL-17, IL-21 and IL-22. Differentiation into Th17 cells depends on TGF-β, IL-6, IL-21 and IL-23, but the timing and relative strength of each can significantly alter the resulting Th17 phenotype. Initial priming in the presence of IL-6 and TGF-β is thought to initiate polarization through STAT3-mediated induction of RORγt, the master transcription factor of Th17 cells. RORγt promotes IL-17 and IL-21 expression, where IL-21 feeds back onto Th17 cells to amplify STAT3 signalling and stabilize RORγt. The IL-23 receptor is also up-regulated via RORγt activity, and therefore IL-23 is an important factor in strengthening Th17 function later in differentiation[16]. Although IL-23, in conjunction with IL-1β, can bypass the need for TGF-β to promote pathogenic Th17 induction[17].

T helper 2 cells

Th2 cells are required to expel large multicellular pathogens, mainly parasitic worms known as helminths, via amplification of the “type 2” response, but are also strongly linked to disorders such as asthma, allergy, dermatitis and ulcerative colitis[18]. The defining characteristics of Th2 cells are expression of the transcription factor GATA3 and cytokines IL-4, IL-5 and IL-13. However, since their

discovery in 1986, the primary signals that instigate Th2 differentiation *in vivo* have remained elusive[18,19].

The cytokines IL-4 and IL-2, which induce the nuclear translocation of STAT6 and STAT5, respectively, are sufficient for *in vitro* polarization[1]. *In vivo*, in contrast to Th1 and Th17 differentiation in which polarizing cytokines are provided by DCs, the initial source of IL-4 has yet to be identified[18]. Th2 cells themselves are the most potent source of IL-4 in the lymph node, implying an earlier signal is likely needed. Accordingly, Th2 cells can arise in IL-4 deficient animals[20,21]. DCs however are still essential for Th2 activation, and conditioning of DCs with helminth-products can result in Th2 priming, suggesting the possible expression of alternative co-stimulatory receptors on DCs that drive Th2 differentiation[22,23]. OX40L and the Notch ligand Jagged 1 expressed on DCs have been identified as possible candidates that promote Th2 development[24,25], but it is contentious whether or not these signals are Th2 specific or simply provide universal co-stimulatory signals to all T cells[26,27].

T follicular helper cells

T follicular helper (Tfh) cells are a population of $CD4^+$ cells that move into the germinal centres (GCs) once activated [28]. Their salient function is to support B cell activation, class-switching and differentiation. Whether Tfh are a distinct lineage of $CD4^+$ subset has been debated. Tfh cytokine production frequently mirrors that of the parallel T effector (Teff) response[29,30], and several reports have

demonstrated Teff to Tfh conversion, and vice versa [31–33]. However, Tfh can be defined by a distinct combination of surface markers (PD-$1^{Hi}$$ICOS^{Hi}$$CXCR5^{+}$) and express their own distinguishing transcription factor, BCL-6[28]. The main function of BCL-6 appears to be suppressing factors that promote other Teff subsets, such as T-bet, RORγt and GATA3[34,35]. BCL-6 deficiency completely inhibits the differentiation of Tfh *in vivo* but has little impact on other subsets suggesting Tfh may not be critical for acute T cell responses. They are, however, critical to GC formation and the humoral response, making them an important consideration in vaccine development[28].

Regulatory T cells

As opposed to Teff subsets, regulatory T cells (Tregs) function to suppress aberrant or unwanted immune responses[36]. Treg differentiation can occur during thymic development, or extra-thymically in peripheral tissues. Thymic Treg differentiation is dependent on high affinity interactions between self-pMHC complexes during thymocyte selection, but not so high as to induce apoptosis. Peripheral Tregs, conversely, are differentiated during naïve T cell activation with weak co-stimulation and signalling from the cytokine TGF-β. Tregs are identified by Foxp3 expression, which is critical for their suppressive capabilities. Deletion of Foxp3 in adult mice causes death within 2 weeks due to aberrant immune cell expansion and activation of self-reactive T cells, illustrating the importance of both Foxp3, and the presence of Tregs in immune homeostasis. Tregs employ several mechanisms to control the Teff response, mainly the secretion of suppressive

cytokines such as IL-10 and TGF-β, and the expression of co-inhibitory receptors such as CTLA-4[37]. Although these mechanisms are non-specific, it has also been postulated that Tregs are activated to optimally regulate particular Teff subsets by mirroring the expression of canonical Teff transcription factors, possibly by allowing Tregs to adopt similar migration patterns as the cells they are targeting for suppression[18,38].

1.1.5 The "second-touch" hypothesis

Antigen-presentation to T cells is well-characterized in the lymph node, however the events occurring during Teff cell entry into peripheral tissue are not as well understood[39]. Once migrated to the peripheral tissue, activated T cells are known to be further instructed by both TCR-dependent and independent signals, though the consequence of these on T cell function are unclear[39,40]. The "second-touch" hypothesis affirms full Teff function is not gained until subsequent secondary antigen recognition after leaving the lymph node. Support for the second-touch hypothesis has been gathered mainly in a Th2 setting, where early work showed that IL-4 transcription and translation are temporally separated[41]. Recent evidence now suggests that Th2 cytokine production is limited in the lymph node, but robust at tissue sites[42,43]. Hence, according to the second-touch hypothesis, activated T cells in the lymph node may be considered "primed" but not yet differentiated effectors[39].

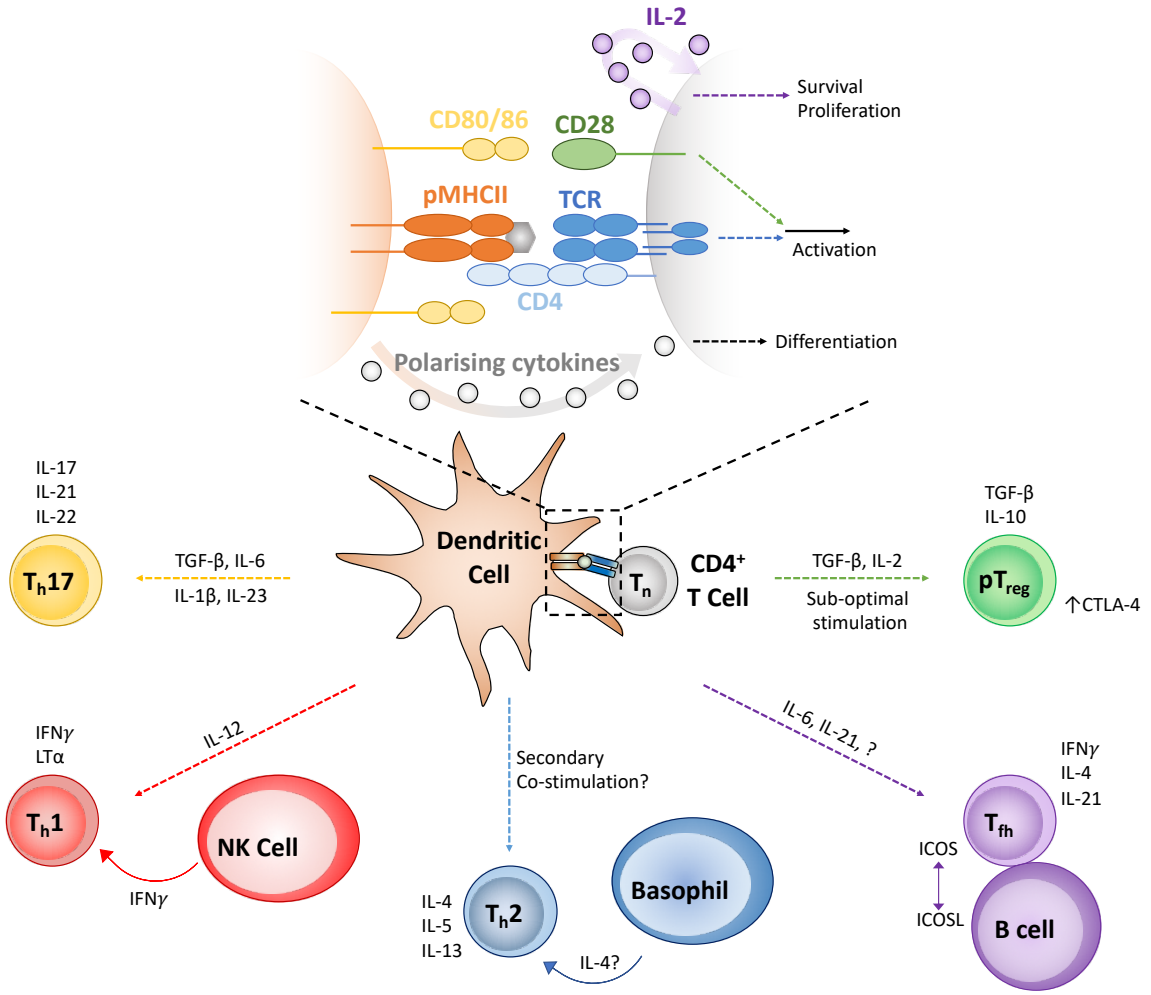

Figure 1.1 | Overview of CD4$^+$ T cell differentiation

Presentation of processed antigenic-peptide by DCs to naïve CD4$^+$ T cells triggers activation through T cell receptor signalling. Co-stimulation through CD80/86 interactions with the CD28 receptor amplifies TCR signalling to support complete activation. Activated Teff can become one of several subsets depending on additional co-stimulatory signals and cytokines present. Th1, Th2, and Th17 cells are the most defined subsets that migrate and produce cytokine at the effector site. Tfh prolong residence in the lymph node to support B cell function. Treg can be thymically derived or activated in the periphery and suppress immune responses to prevent autoimmunity or excessive immune-driven pathology.

1.1.6 Contraction: death, memory, and exhaustion

Primary activation leads to rapid proliferation and expansion of the effector T cell pool. Following the successful resolution of infection, the large pool of cytokine-producing Teff cells is no longer of use and may be detrimental if maintained. Therefore, once an infection is cleared, the vast majority of activated T cells engage pathways for cell death[44]. The mechanisms controlling this self-limiting contraction phase are poorly understood but may be a combinatorial result of reduced antigen-exposure, growth factor withdrawal, and extrinsic “death” signals provided by other cells.

A small proportion, less than 10%, of T cells activated during the primary response survive beyond the resolution of infection as a long-lived memory population[45]. Memory T cells are maintained in quiescence until subsequent reactivation upon secondary antigen encounter. Though quiescent, memory cells are already metabolically and transcriptionally conditioned for activation, and therefore provide a more rapid and robust response in the event of a recurring infection[46]. Memory cells exist as heterogenous subsets defined by their location and response to secondary challenge[45]. Central and effector memory $CD4^+$ T cells circulate throughout the body, with central memory cells moving mainly between lymphoid organs and effector memory cells transiting through the blood, to and from peripheral tissues. Conversely, resident memory T cells maintain localisation in the tissue and do not recirculate.

Certain pathogens are adept at hiding from, or manipulating host immunity, and in these scenarios the adaptive response may be unable to adequately dispel infection. During chronic infection, activated T cells can be continually exposed to pathogen-derived antigens, but decline in their ability to proliferate and produce cytokine upon recognition. This less-functional state, despite the constant presence of foreign antigen, has been termed T cell exhaustion[47].

1.2 Type 2 immunity in disease

Type 2 immunity, synonymously referred to here as the Th2 response, has a dichotomous role in global health and in disease. Large parasitic worms, known as helminths, are the primary pathogenic inducers of type 2 immune responses, thought to be beneficial for host resistance. Helminth infection, however, has been largely eradicated in developed, economically wealthy areas of the world. These regions instead face an increasing prevalence of disease characterized by unwanted Th2 responses, most predominantly exemplified by atopic disorders, including allergy and certain forms of asthma[48].

1.2.1 Epidemiology of helminth infection

Helminths are one of the most prevalent causes of infectious disease in the world. Recent figures estimate up to 2 billion individuals are affected by helminth infection, predominantly in poor regions of the tropics and sub-tropics[49]. Helminth infection also occurs in livestock world-wide, providing an increasing constraint on

agricultural productivity as the globe is becoming more reliant on livestock consumption in light of dwindling natural resources[50]. Although infection is not acutely life-threatening, it is typically long lasting and can cause persistent debilitation through malnutrition and physical disability[49]. Though, mortality is a particular risk for pregnant mothers or their offspring. Mortality can also result from parasite growth blocking critical organ systems or indirectly by altering protective immunity against co-endemic malaria and HIV[49,51]. The intensity and prevalence of infection is highest in children of low-income regions, causing restricted growth, physical fitness and cognitive development. Therefore, the burden of helminths has severe individual and societal implications, and is ultimately a significant factor in maintaining developing regions in a continual state of poverty[49].

Helminth species are diverse in their transmission kinetics and life-cycle[52]. They are classified under three taxonomic groups: cestodes, trematodes and nematodes. The nematodes include the highly prevalent soil-transmitted helminths, as well as the more debilitating filarial parasites. Soil-transmitted nematodes, including the roundworm *Ascaris lumdricoides*, the hookworms *Necatar americanus* and *Ancylostoma duodenale*, and the whipworm *Trichuris trichiura*, account for over two-thirds of worldwide human helminth infection[49,52]. Once in a host, soil-transmitted helminths settle in the intestine where they fully mature and produce eggs, and in the absence of treatment, take up long-term residence for chronic infection. Eggs ejected with host faeces hatch into infective

free-living larvae (with the exception of *Trichuris sp.*) to form a new reservoir for transmission through skin contact or direct ingestion (Fig. 1.2).

In humans, resistance to helminths positively correlates with indicators of type 2 immunity[52], while experimentally, Th2-dependent protection as been affirmed using murine models[53,54]. The chronic nature of helminth infection, together with the detection of immunosuppressive cytokines and regulatory immune cell populations in patients, is highly suggestive of active immune manipulation by the parasites to neutralize Th2 responses[52]. Anthelmintic therapies are available for the treatment of helminthiasis, but drug-resistance is developing in ruminants and in humans, and they are not effective at preventing reinfection[49,52,55]. Thus, the development of vaccination strategies is needed. Currently our limited understanding of both Th2 biology, and the mechanisms participating in helminth-mediated suppression have hampered progress towards this goal[56].

1.2.3 Atopic disease

Next to helminth infection, atopic, or allergic disease is the most prominent occurrence of Th2 responses. Atopic diseases are estimated to affect up to 20% of people in developed countries and provides a significant burden for health care systems[57,58]. These include atopic dermatitis, food allergy, allergic asthma and rhino-conjunctivitis (or “hay fever”). Additionally, an unprecedented rise has been observed in allergy related disease to the extent it has been labelled an “epidemic”[48].

The cause of allergic disease relies on a diverse set of criteria involving genetic predisposition and environmental risk factors that vary from individual to individual[48,58]. Both innate and adaptive arms of the immune system are involved in driving atopy. Pathogenesis involves previous sensitization of the immune system to environmental antigens, which leads to IgE antibody production, and the subsequent activation of innate mast cells and basophils that produce cytokines, histamine, lipid mediators and proteases that drive allergic symptoms[59]. Pathological memory Th2 cells are further developed that maintain or amplify disease[58,60,61]. Experimentally this has been demonstrated in mice. For instance, sensitization and challenge of mice with house dust-mite (HDM) extract is a good illustration of allergic mechanisms, as the Der p 1 protein from HDM is also a common allergen that triggers airway reactions in humans[62].

1.2.4 Old friends, new diseases

Human helminth infection has been recorded dating back to ancient Egypt, emphasizing the co-evolution between parasites and host[49]. The marked increase in allergic disease in the last 3 decades has coincided with the reduction of helminthiasis in the western world, yielding the hypothesis that helminths have a potentially beneficial role in mitigating host inflammatory disease[48]. However, only a select few examples have demonstrated a causation between infection and improved disease prognosis of host inflammatory disease. Most well-known, a small cohort of multiple sclerosis patients in Argentina were followed that had naturally acquired helminth infection[63]. Compared to matched, uninfected patients,

those with helminths remained in remission with less frequent relapse. Importantly, the anthelmintic treatment of a subset of patients resulted in increased symptoms of relapse[64].

The beneficial potential of helminths elucidated from animal models has sparked intense interest in the area of helminth therapy in humans, though clinical trials have yet to demonstrate a clear benefit from controlled, live infection[65]. In light of these efforts, experts in the field have reiterated that helminths are not to be forgotten as true professionals in parasitism, and are not mutualists[66]. There is, however, an evident immunological relationship between helminths and their longevity in hosts, which may be harvested for therapeutic potential by identifying the key molecular mediators of regulation[52,67].

1.3 Experimental models of helminth infection

Mechanistic insight into the type 2 immune response during helminthiasis has been gained using murine models of infection that recapitulate several aspects of disease in humans. *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis* are two of the most thoroughly studied helminths in mice and have yielded much of our current understanding of immunity to helminths.

1.3.1 *Heligmosomoides polygyrus*

The intestinal nematode *H. polygyrus* is a natural parasite of wild mice that has been adopted for experimental laboratory use. Phylogenetically, it is related to the human hookworms[68]. Susceptible mouse strains endure *H. polygyrus* infection for several months, mirroring the chronicity of human infection[68]. This makes it a useful model for interrogating the immune pathways that suppress or promote resistance.

H. polygyrus undergoes several stages of maturation in the host small intestine (Fig. 1.2)[68,69]. In an experimental setting, larval stage parasites are given to mice by oral gavage. Within 24 hours, larvae burrow through the intestinal barrier and continue development beneath the submucosa. After a week of moulting, adult parasites re-emerge into the lumen, coil around villi, and begin mating and reproduction. The success of infection, and the viability of parasites, can be enumerated by the recovery of live worms in the small intestine or the egg output in faecal matter.

H. polygyrus is equally used to study infection in the context of immune-regulation as much as it is used to study the dynamics of type 2 immunity[68]. Though infection is characterized by potent Th2 $CD4^+$ T cell expansion, it also expands Treg populations that persist beyond the peak of the Teff response[70,71]. Recent discoveries have also identified secreted proteins from *H. polygyrus* that are functionally suppressive of the host immune response[72–74]. Therefore, *H.*

polygyrus is a valuable model for understanding the balance between Th2 induction and immune regulation.

1.3.2 *Nippostrongylus brasiliensis*

N. brasiliensis in an acute model of helminth infection that has been adapted to mice, though its natural host is primarily the rat[69]. The transmission and development of *N. brasiliensis* resembles that of human hookworms, though the parasite itself is only distantly related[69,75]. Infection is initiated by sub-cutaneous injection of larval parasites (Fig. 1.2). Movement into blood vessels following infection carries larvae towards the lung, which they enter and reside in for up to two days to moult, before being coughed up and swallowed into the digestive tract. Once in the gut, worms undergo a final moult to become mature adults and weakly attach themselves to the wall of the small intestine. Mating occurs after 6 days from initial inoculation; however, once in the intestine, host defences take only 3-5 days to clear adult worms[69].

In contrast to *H. polygyrus*, the immune response elicited by *N. brasiliensis* is a complete and more potent Th2 response[69,75]. The motility of *N. brasiliensis* within the host during its life cycle also provides opportunities to assess the progression of the immune response in multiple, easily accessible sites, including the skin, lung and intestine. Although infection is limiting in healthy hosts, it can last several weeks in immune-deficient mice. Therefore, worm burden can provide an effective

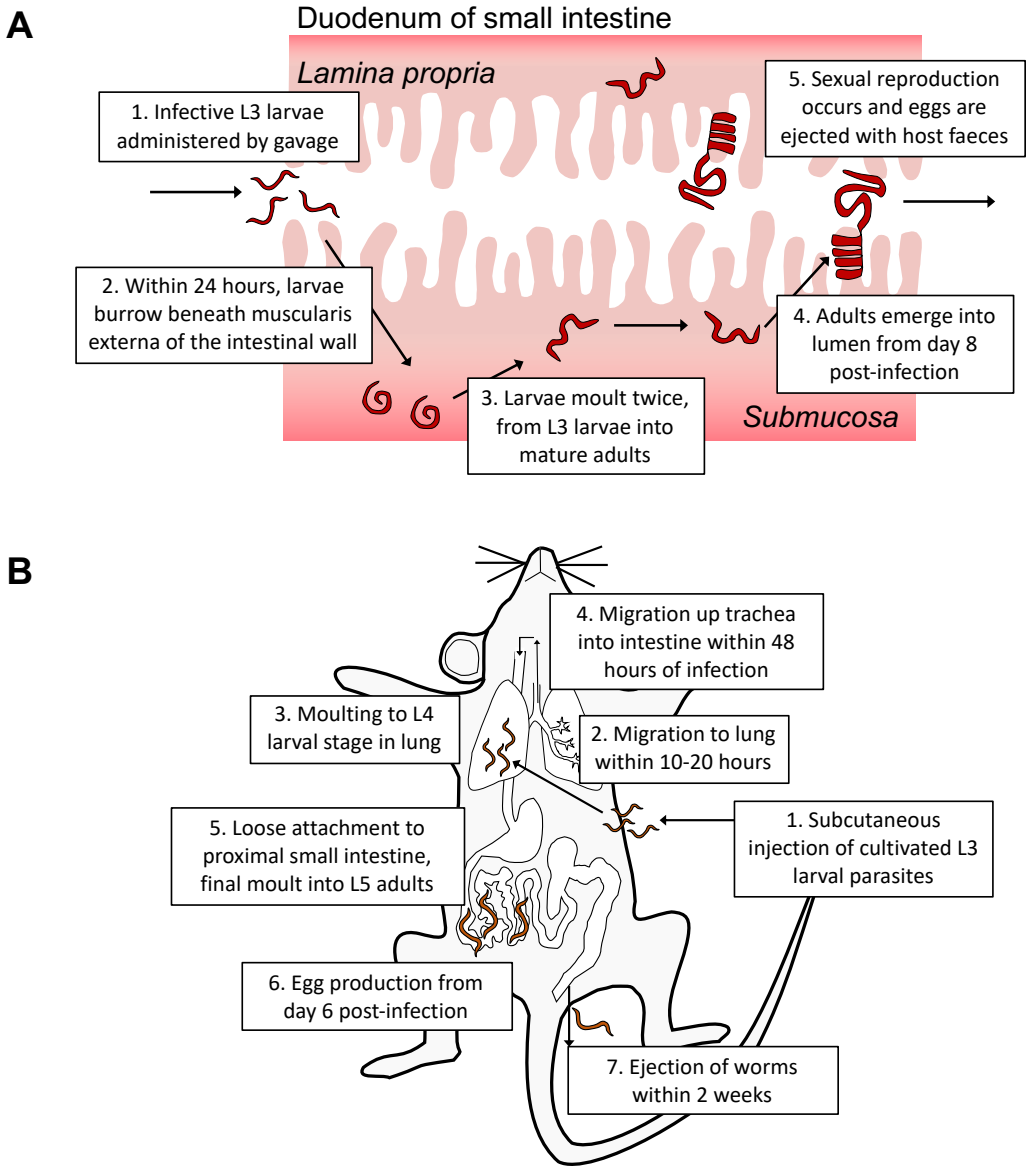

Figure 1.2 | Experimental models of helminth infection

A, Lifecycle of *H. polygyrus* infection. Parasites are infected by oral gavage, and develop fully within the small intestine, where depending on the mouse strain, they will remain for up to several months. Adapted from Reynolds and Maizels[68] **B**, Lifecycle of *N. brasiliensis* infection in mice. Larvae are injected into the skin, migrate to the lung where they undergo one moult before reaching the intestinal tract via the trachea. Full maturity is reached in the small intestine, but worms are rapidly expelled in wild-type strains. Adapted from Camberis, Le Gros and Urban[69].

readout to test the importance of different aspects of type 2 immunity in the context of infection[69].

1.4 Anti-helminth immune responses

Broadly speaking, resistance to helminths requires type 2 immunity. However, the responses elicited by infection varies between different species of helminth. The key cellular players, and their functional requirements for expulsion, are not always universal. Despite this variation in immune requirements, there are several common aspects of immunity that are observed across species. The subsequent description of anti-helminth immunity is an integrated summary of our current understanding in respect to soil-transmitted nematodes, largely in respect to the model organisms *H. polygyrus* and *N. brasiliensis.* (Fig. 1.3)

1.4.1 Detection and innate activation

The epithelium is the first point of contact between parasite and host. Hence epithelial cells provide the initial signals to alert the host to a potential invasion[76]. Upon sensing helminths, or subsequent to barrier breach, epithelial cells release alarmin cytokines, in particular IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), that prime the immune system. IL-25 has recently been found to be selectively produced by specialized epithelial tuft cells that sense changes in luminal metabolites in the intestine[77]. IL-33, however, is constitutively expressed

and stored within epithelial cells, but only released during cellular stress or damage, and signals back to the local epithelium to also produce TSLP[76,78].

A key role of IL-25 and IL-33 early in response to infection is to activate type 2 innate lymphoid cells (ILC2s), which provide an early source of canonical Th2 cytokines[79]. IL-4, IL-5 and IL-13 from ILC2s together promote the development and recruitment of eosinophils and basophils, induce goblet cell hyperplasia and mucus secretion, activate and expand mast cells, and enforce the polarization of alternatively activated macrophages (AAMs). Despite the contribution of ILC2 to the overall type 2 immune response, they are insufficient to expel helminths in the absence of adaptive immunity[80,81]. However, host resistance to infection is reduced in the absence of ILC2s, particularly against *N. brasiliensis*[80–82]. Recent work has additionally described important dialogue between ILC2 and Th2 cells needed for optimal anti-helminth immunity[80,82,83].

1.4.2 Induction of adaptive immunity

Expulsion of helminths requires the activation of an adaptive, $CD4^+$ Th2 response. Dendritic cells are critical for the activation and polarization of Th2 cells[18]. Characterizing DC subsets, and their ability to prime specific responses, has been an intense area of recent research. It is now understood that phenotypically distinct DCs activate different $CD4^+$ T cell subsets, although the signals promoting Th2-priming DCs, and the unique mechanisms these DCs employ to specifically activate Th2 cells, have yet to be fully elucidated[84–87]. DCs are activated in

response to their detection of highly conserved pathogen-derived molecules via pattern-recognition receptors, namely toll-like receptors, nod-like receptors or C-type lectin receptors[88]. Interestingly, Th2-priming occurs independently of MyD88, the critical adapter protein immediately downstream of toll- and nod-like receptors[89]. Indeed, the only helminth molecule currently identified to directly activate Th2-inducing DCs is omega-1, a small glycoprotein from the parasite *Schistosoma mansoni*, which binds the mannose receptor, a C-type lectin receptor, via surface glycans[88,90].

DCs also express receptors for epithelial alarmins. TSLP in particular has been shown to condition DCs for polarizing Th2 responses[24]. The necessity of TSLP *in vivo* is redundant, however, as both *H. polygyrus* and *N. brasiliensis* elicit Th2 responses in the absence of TSLP signalling[91]. IL-33 has been shown to induce DC maturation, and co-culture with IL-33 activated DC can induce T cell production of Th2 cytokines *in vitro*[92]. Equally, it has also been shown that IL-33 promotes DC-mediated Treg induction[93]. It should be noted that the role of IL-33 specifically on DCs has yet to be fully assessed *in vivo* during helminth infection. Altogether, the signals instructing DCs to prime Th2 responses are still uncertain, but depending on the context, may be a combination of pattern recognition receptor signalling and alarmin sensing. Once activated and carrying antigen, DCs migrate to the draining lymph node to prime the T cell response. The migration of DCs from the tissue to the lymph node may be induced, at least in part, by ILC2-produced IL-13[94].

1.4.3 Th2 development in the lymph node

Helminth antigens are presented as processed peptides by DCs to naïve T cells in the draining lymph node to elicit both Th2 and Tfh responses[30,32]. A recent study suggested that antigen presentation and T cell activation may occur proximal to the B cell follicle by CXCR5-expressing DCs[95]. Interestingly, this fits with work from the same group that later reported Th2 differentiation progresses from Tfh cell precursors in HDM induced allergic-asthma[31]. However, the reverse, that Tfh arise from Th2 precursors, has also been reported during *S. mansoni* infection[32]. The exact relationship between Tfh and Th2 in the lymph node remains to be delineated fully, but populations of both cells are readily detected following DC migration to the lymph node. Tfh cells remain in the follicle and promote B cell activation and class-switching to produce IgG neutralizing antibodies and IgE for the activation of mast cells and basophils[79]. Th2 cells leave the lymph node to migrate to the infected site and enforce defensive mechanisms for parasite resistance there.

It is currently unknown how Th2 cells are polarized in the lymph node. IL-4, which is sufficient for Th2 induction *in vitro,* is not produced by DCs, and although other potential sources of IL-4 have been identified, namely basophils, resistance to helminth infection is maintained in the absence of IL-4 leading to the notion that IL-4 is dispensable for the development of adaptive responses[18,20,21,54,79]. Several co-stimulatory receptors have been tested for their role in Th2-priming, but so far none have proven to be critical, or unique, in activation of Th2 cells[18]. Surprisingly,

given its well-established role in T cell activation, CD28 was also found to be dispensable for clearing *H. polygyrus* or *N. brasiliensis* infection[96,97]. One hypothesis, which continues to accumulate supporting evidence, is that Th2 differentiation is a result of weak TCR signalling. Low-dose antigen stimulation of naïve T cells with a transgenic TCR is associated with a Th2-bias *in vitro* and *in vivo*[98–100]. Furthermore, it has recently been shown that CD4-specific deletion of E3-ubiquitin ligases that promote the degradation of the phosphatase SHP-1 causes weakened TCR signalling in mice, and uncontrolled Th2-mediated inflammation[101].

1.4.5 Tissue-based Th2 amplification

Multiple reports now support the second-touch hypothesis in the context of Th2 cells. T cells activated in the lymph node migrate to peripheral tissue sites where they encounter several professional and amateur APCs, including macrophages, ILC2, eosinophils and basophils[102]. Antigen presentation in peripheral tissues has not been thoroughly studied in general, though a prominent role has recently been ascribed to ILCs. During *N. brasiliensis* infection, ILC2 have been shown to express MHCII in the lung that can promote local Th2 expansion and cytokine production[82]. Co-stimulatory OX40L, as well as co-inhibitory PD-L1, on ILC2 have further been identified as factors promoting tissue Th2 development[83,103]. Interestingly, antigen presentation by ILC3 seems to have an opposite, inhibitory effect on commensal-specific T cells in the intestine[104]. Terminal Th2 differentiation is also enforced through alarmin signalling in the inflamed tissue. In

particular, IL-33 signalling through its receptor, ST2, promotes TCR-independent Th2 cytokine production[42,43]. Altogether, in the context of Th2 differentiation, studies support a model in which cells primed in the lymph node do not acquire full effector function until arrival at the initial site of infection or inflammation.

1.4.5 Mechanisms of helminth expulsion

Tissue-recruited Th2 cells produce cytokines that amplify the early innate response to facilitate worm expulsion[79,81]. Helminth clearance during primary infection relies centrally on physiological changes in the intestine to promote the physical removal parasites from their niche[76]. This is mainly achieved through the actions of IL-4 and IL-13 that directly and indirectly alter the epithelium. Cytokine signalling induces rapid proliferation and shedding of epithelial cells to uproot embedded parasites[105]. Goblet cell hyperplasia is induced to secrete mucus, which both increases the barrier thickness and inhibits worm motility and vitality[106,107]. Mast cells attracted into the tissue by Th2 cytokines also aid epithelial shedding by the release of proteases that weaken tight junctions[108]. If parasites are successfully ejected from the epithelium, they are further displaced by IL-4 and IL-13 induced smooth muscle contractions and peristalsis[109].

Expulsion may also be supported by the direct attack against parasites with toxic mediators released mainly by granulocytes[76,110]. The singular role of IL-5, which is robustly induced during infection, is to recruit eosinophils. Eosinophils have consistently demonstrated an ability to directly kill worms *in vitro*, though results *in*

vivo are more variable[76]. Overexpression of IL-5 in some scenarios, including *N. brasiliensis* infection, can promote resistance, though eosinophil deficiency has demonstrated little impact on the outcome of helminth infection[53,111,112].

In a similar token, AAM display an ability to restrict larval parasite fitness through mechanisms that require direct binding, but *in vivo* it is uncertain what role they play[110,113]. In *H. polygyrus* infection, granulomas form around parasites residing in the submucosa during the larval stage, which consist predominantly of macrophages, as well as neutrophils and eosinophils[68]. Indeed, enhanced granuloma formation is associated with resistance, although the interactions responsible are unknown. During initial infection, it appears that the most critical role of AAMs is to orchestrate tissue repair by suppressing the T cell response and promoting collagen deposition through arginase-1 expression[110,114,115]. Resistance to a secondary challenge infection, however, appears to depend critically on IL-4Rα stimulated macrophages[114,116].

1.4.6 Memory responses to secondary challenge

Re-infection with helminths, unlike primary infection, is rapidly cleared in mice. Resistance to secondary infection depends on successful activation of the Th2 response during primary infection[116]. Memory Th2 cells are characterized by constitutive expression of the IL-33 receptor, ST2, which promotes rapid TCR-independent activation upon signalling[42,117]. Therefore, the epithelium can promote a robust T cell response quickly after helminth detection.

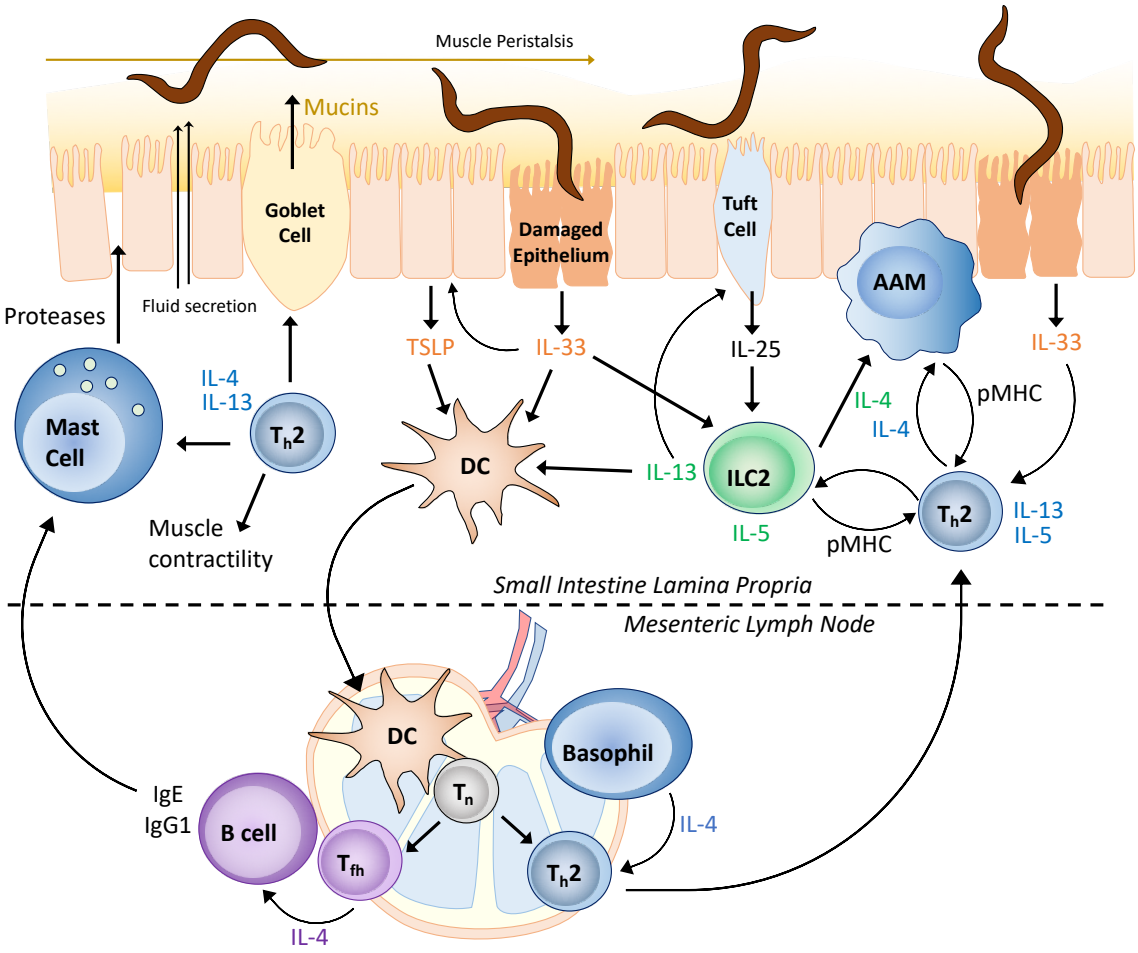

Figure 1.3 | Overview of type 2 immunity against intestinal helminths

Immune responses against helminths are initiated by epithelial cells that secrete alarmins. Alarmins activate type 2 innate lymphoid cells (ILC2) and act on DCs to promote Th2-priming after migration to the lymph node. Activated Th2 cells emigrate back to the tissue, while Tfh promote IgG1 and IgE class-switching by B cells. In the tissue, secondary antigen encounter and alarmins stimulate peripheral Th2 cytokine production. Th2 cytokines from T cells and ILC2 promote eosinophil, basophil and mast cell recruitment and activation, along with secreted IgG and IgE, as well as alternatively activate macrophages. Innate cells produce toxic mediators that may directly damage worms, but also alter intestinal physiology to “weep and sweep” parasites away. Th2 cytokines further act directly on the epithelium to promote proliferation and shedding, and signal to the underlying muscle to trigger peristalsis and move adult worms away from their niche. Adapted from Loke and Harris[79] and Maizels, Hewitson and Smith[118].

The response to secondary infection is highly dependent on direct helminth-cidal activity by macrophages. Macrophage recruitment to *H. polygyrus* granulomas is enhanced during secondary infection, which can be recapitulated in primary infection by transferring helminth-primed T cells[116]. Secondary *N. brasiliensis* infection similarly recruits AAM that directly kill lung-migrating larvae[114]. The humoral response during primary infection also provides an important role in secondary protection via IL-4Rα independent alternative activation of macrophages[119]. Helminth-specific antibodies provide a trapping mechanism for tissue-invading larvae, while simultaneously promoting attachment and larval destruction by macrophages via activating Fc receptors. Hence, the main role of the memory T cell response appears to be priming the innate response to mediate early destruction of invading larvae, preventing the establishment of infection.

1.4.7 Helminth regulation of immunity

In mice, and humans, increased suppressive cytokines and tolerogenic cell populations can be detected during helminth infection[52]. With the current interest in helminth therapy, there is a drive to understand the molecular basis for immune regulation during infection. Mainly, this has been studied by characterizing the total excreted and secreted products (ES) from parasites in culture. This approach so far has led to the discovery of up to 50 parasite-derived molecules, mostly proteins, from several different species, with immuno-modulatory properties[67]. The targets of modulation are also diverse, including surface receptors and intracellular signalling pathways, and these molecules impact all stages of the immune

response, from initiation to resolution and repair. Many more modulators are sure to be revealed as technologies improve for high-throughput identification and screening. The prospect of adopting these molecules into clinical use for inflammatory disorders is promising but requires an in-depth understanding of their full impact on host health and immunity, which is still incomplete.

1.5 T cell immunometabolism

Cellular metabolism has been studied for over a century, yet only in the last decade has its importance been fully appreciated in immunology, yielding the integrated field of immunometabolism. Seminal studies in the field have discovered metabolic activity in immune cells is highly dynamic and context dependent, and importantly, underpins several aspects of immune function[120–122]. Furthermore, the rapidly growing volume of literature in immunometabolism now allows metabolic characterization to be used as a tool that can guide interpretation of immune cell history, environment and function. It is now also becoming evident that multiple widely prescribed therapies indeed act as metabolic manipulators within the immune system, including rapamycin for the treatment of transplant rejection, dimethyl fumarate for psoriasis and multiple sclerosis, as well the Nobel prize-winning checkpoint blockade antibodies used for anti-cancer therapy[123–125]. Immunometabolism, therefore, has already provided valuable insights into the workings of the immune system, and has retroactively proved its therapeutic potential.

1.5.1 Core metabolic pathways

Metabolic pathways are either catabolic or anabolic. Anabolism is the synthesis of complex molecules from simple molecular building blocks and requires energy, whereas catabolism is the reverse, and yields smaller metabolites to be used for energy generation or building blocks in anabolic reactions. A cell's metabolism can then be characterized as primarily anabolic or catabolic as the dependence on different pathways shifts according the cell's needs. The so-called "core" metabolic pathways consist of the catabolic breakdown of glucose, glutamine and fatty acids that generate cellular energy, as well as precursors for several ancillary, anabolic, biosynthetic pathways (Fig. 1.4).

Oxidative phosphorylation

Ultimately, the energetic status of a cell is determined by adenosine triphosphate (ATP) production through catabolic reactions. ATP is maintained mostly by the oxidation of glucose, glutamine or fatty acids[126]. The breakdown of these metabolites fuels the tricarboxylic acid (TCA) cycle in the mitochondria, yielding the reducing intermediates NADH or $FADH_2$, that donate electrons to the electron transport chain (ETC). As electrons are moved along conducting protein complexes on the inner membrane, the controlled transport of hydrogen atoms into the mitochondria is coupled to ATP generation. This process requires oxygen as the terminal electron acceptor, which causes the formation of reactive oxygen species (ROS), that are quickly bonded to hydrogen to form water. This process is called oxidative phosphorylation (OXPHOS).

Glycolysis

Glycolysis, the breakdown of glucose, feeds the TCA cycle and OXPHOS through glucose-derived pyruvate. There are at least 13 characterized glucose transporters, though GLUT1 is the most central transporter used by T cells[127]. Once intracellular, glucose is broken down through a 10-step enzymatic process, beginning with phosphorylation of glucose by hexokinase II (HK2), followed by the eventual splitting of the 6-carbon ring into two 3-carbon molecules that are finally converted to pyruvate by pyruvate kinase (PKM2). Pyruvate is then transported into the mitochondria for entry into the TCA cycle. One molecule of oxidized glucose results in a maximum theoretical yield of 38 ATP.

In addition to fuelling the TCA cycle, glycolysis has several branch points that link to important biosynthetic pathways[126,128]. The pentose-phosphate pathway (PPP) branches off immediately after glucose has been phosphorylated by HK2 and provides the sugar backbone for nucleotide synthesis. The product of the second step of glycolysis reacts with glutamine to additionally fuel hexosamine biosynthesis, essential for the post-translational modification of proteins. Later in the glycolytic pathway, the intermediate 3-phosphoglycerate can be diverted to serine biosynthesis, important for nucleotide and amino acid formation. Therefore, a molecule of glucose does not always end up fuelling the TCA cycle and can instead support one of several anabolic pathways.

Fatty acid metabolism

Fatty acids (FA) are essential precursors for phospholipid membranes, are key molecules in signalling and post-translational modifications, and can also provide a highly efficient source of fuel for ATP generation[126]. FA can be broken down through fatty acid oxidation (FAO) in the mitochondria or synthesized in the cytoplasm via fatty acid synthesis (FAS). As FAO is catabolic and FAS is anabolic, the activities of each pathway are counter-regulatory to the other[129]. These pathways are frequently discussed in relation to palmitate, a 16-carbon long-chained FA, one the most highly detected free-FA in human serum[126,130]. Complete oxidation of one palmitate produces over 100 ATP[126].

FAO can be achieved using either extracellularly acquired, or cell-intrinsic FA. The main route of cellular entry for environmental FA is through several FA transporter proteins (FATPs) as well as the scavenger receptor CD36[131]. FATPs display tissue tropism, but CD36 is ubiquitously expressed, and has demonstrated critical functions in immunity[131,132]. Cell-intrinsic lipolysis provides an additional source of FA from recycled organelle membranes, or lipid storage bodies, already within the cell. Intracellular FA binding proteins (FABP) act as cytoplasmic chaperones for internalized FA to coordinate diverse lipid-related processes[133]. Lipids targeted for FAO enter the mitochondria through carnitine-palmitoyltransferase-1α (CPT1α) and, through the process of β-oxidation, are converted to multiple units of acetyl-CoA, which can then shuttle into the TCA cycle.

FAS, the opposite process to FAO, forms long-chain FA by linking together acetyl-CoA building blocks. FAS begins with shunting of the TCA product, citrate, into the cytoplasm from the mitochondria. There, citrate is converted back to acetyl-CoA by ATP citrate-lyase (ACLY)[129]. Acetyl-CoA is further converted into malonyl-CoA by acetyl-CoA carboxylase 1 (ACC1, encoded by *ACACA* gene), before being elongated into long-chain FA by the consecutive addition of acetyl-CoA molecules, through the enzymatic activity of fatty acid synthase (FASN).

Glutaminolysis

Glutamine is the most abundant amino acid in serum and is therefore a valuable substrate for several cellular functions[134]. Several amino acid transporters can be expressed for glutamine uptake, namely ASCT2 and SNAT1 and 2[135,136]. Major roles of glutamine include protein synthesis, nucleotide synthesis and post-translational modifications[126,134]. Metabolically, glutaminolysis can fuel OXPHOS through the direct conversion to the TCA metabolite α-ketoglutarate, once transported into the mitochondria. The catabolic breakdown of glutamine is therefore an additional method for ATP generation, which can maintain cellular energy in conditions where glucose is limiting[137].

1.5.2 Warburg metabolism

As discussed above, glycolysis normally leads to ATP generation via the TCA cycle and oxidative phosphorylation, a process that requires oxygen. However, glycolysis can also proceed in the absence of oxygen to convert pyruvate to

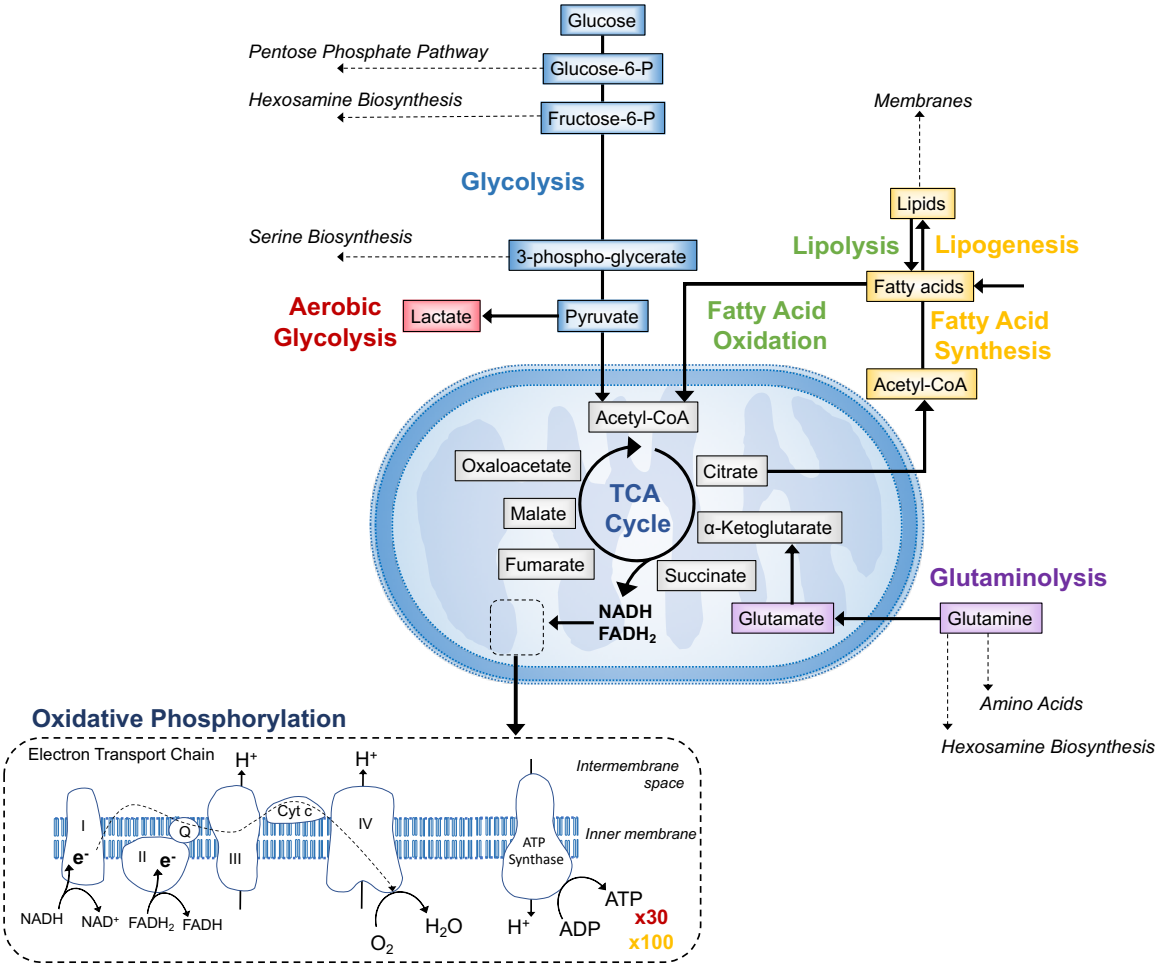

Figure 1.4 | Overview of core metabolism

ATP is a requirement for cellular survival and function. The most efficient method of ATP generation is oxidative phosphorylation, which takes place along the inner membrane of the mitochondria. Electrons for OXPHOS are passed along the ETC before being accepted by molecular oxygen to form reactive oxygen species, which are neutralized by hydrogen atoms to form water. Electrons are derived from intermediates, NADH and FADH2, formed during the tricarboxylic acid (TCA) cycle, which is fuelled by the catabolic breakdown of metabolites, namely glucose, glutamine and fatty acids. Adapted from Buck, O'Sullivan and Pearce[138].

lactate, instead of fuelling mitochondrial metabolism. Classically this is referred to as fermentation, providing an alternative energy source in times of limiting oxygen availability. This pathway is highly inefficient as it produces only 2 net molecules of ATP and the majority of lactate is secreted as waste. However, Otto Warburg, in the 1920s, made an observation that cancer cells in culture had a high propensity to metabolize glucose to lactate, even in atmospheric oxygen[139]. This observation has been termed the “Warburg effect” and, although it is a metabolic cornerstone for cancer cells, it is also frequently observed in immune cells. Even though glycolysis is by nature a catabolic pathway, it is also a backbone for several anabolic biosynthetic pathways. As cancer cells, or immune cells, rapidly grow and divide, they need both the building blocks to synthesize new proteins and membranes, as well as the ATP to sustain these processes. Warburg metabolism, or aerobic glycolysis, produces ATP rapidly, though inefficiently, while freeing intermediates for the creation of molecular building blocks. Therefore, Warburg metabolism may strike a balance between energy generation and biosynthesis, providing a possible rationale for the rapid proliferative ability in cancer and activated immune populations[2,126,128].

1.5.3 Metabolic reprogramming during T cell activation

The metabolic demands of a T cell vary dramatically over its lifespan[121]. Naïve T cells are sustained by a relatively low metabolic activity as they circulate the body seeking antigen. Primarily, they rely on a low rate of OXPHOS maintained by FAO[140]. TCR stimulation with CD28 co-stimulation, however, induces a dramatic

shift in metabolism, favouring glycolysis, to support the newly acquired demands of activation, as cells rapidly grow, divide and develop effector functions[136,141–143] (Fig. 1.5). As Teff transition back into quiescent, resting, memory T cells, metabolism again shifts to favour OXPHOS, but in a way that cells are "metabolically primed" for quick effector function upon restimulation[144,145].

Activated Teff increase both catabolic and anabolic pathways, however, proportionally there is a much greater increase in anabolic metabolism[2,137,146]. T cell activation induces rapid glucose, glutamine and fatty acid uptake[136,142,147]. Though, most notably, instead of fuelling OXPHOS with glucose, recently activated T cells strongly display Warburg metabolism[141]. Increased OXPHOS is instead maintained mostly by glutaminolysis[137]. Both glycolysis and glutaminolysis are highly dependent on CD28 signalling, although interestingly, glucose uptake and not glycolysis increases with increasing titres of anti-CD3[136,141,142]. Presumably, the increase in aerobic glycolysis following complete activation is used to fuel greater biosynthesis, while also providing a quicker, yet less efficient, means to produce ATP, though the reasoning for T cells using aerobic glycolysis is still mostly speculation[2,139]. However, at least *in vitro*, increased glucose metabolism is critical to T activation, as GLUT1 deficiency abrogates glycolysis, cell survival and proliferation following TCR stimulation, as does activation in glucose-deplete media[127,137,146].

Increased glycolytic flux also provides an increased intracellular pool of acetyl-CoA available for use in FAS. $CD4^+$ T cells deficient in ACC1, a rate-limiting enzyme for *de novo* lipogenesis, are unable to form Teff cells, suggesting FAS is a necessary facet of T cell activation[143]. T cells also acquire extracellular FA early after stimulation, which contribute to promoting exit from quiescence, as cells activated in FA-starved conditions have significantly reduced proliferation[147]. The high demand for FA is likely for the supply of rapidly expanding membranes as cells grow and proliferate.

Aerobic glycolysis was very much the focal point of early T cell metabolism studies, but the increase in mitochondrial OXPHOS during T cell activation is also functionally significant. Mitochondrial morphology and composition is dramatically altered following TCR stimulation[148,149]. These changes are critical in promoting cell-cycle progression, as blocking ETC activity with the chemical inhibitor oligomycin stops TCR-driven proliferation[146]. Similarly, knocking out a key component of one of the ETC complexes, complex III, abrogates Teff proliferation and IL-2 production[150]. Furthermore, mitochondrial dysfunction is strongly associated with, and may be an underlying contributing cause of T cell exhaustion[151–153].

Little work has been done on T cell metabolism in the context of $CD4^+$ memory. Nevertheless, it is believed that following the primary response and before they re-encounter antigen, memory cells reduce glucose metabolism, but maintain a high

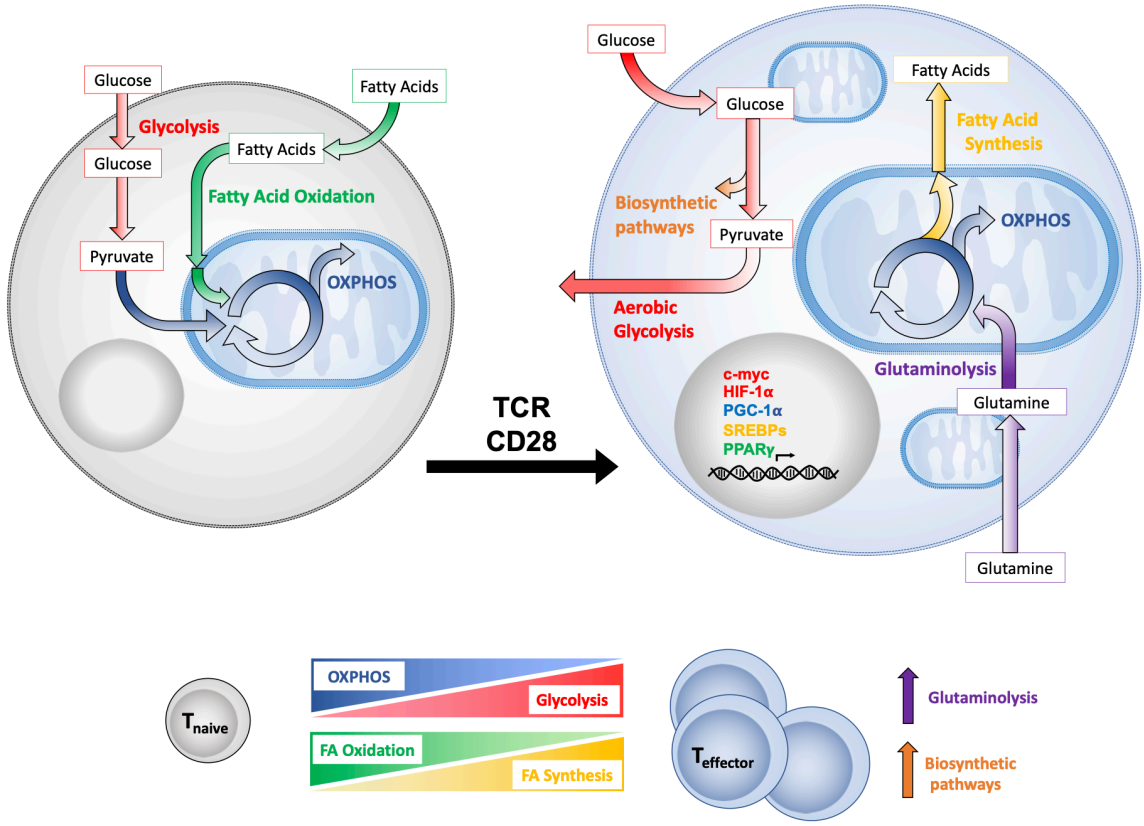

Figure 1.5 | Metabolic reprogramming in T cell activation

T cells switch to a predominantly anabolic metabolism to support activation, proliferation and effector function. Naïve cells maintain a basal level of oxidative phosphorylation dependent on fatty acid oxidation and minimal glycolysis. TCR triggering, combined with CD28 ligation, induces metabolic reprogramming. Activated Teff engage Warburg metabolism, increasing glucose uptake, but secreting the majority of it as lactate. Aerobic glycolysis may be engaged to fuel biosynthetic pathways needed for cellular growth. Fatty acid metabolism is switched from oxidation to synthesis to support membrane growth. Glutaminolysis is upregulated to support mitochondrial metabolism.

rate of FAS[144]. However, FAO is also elevated so that memory cells are engaged in a futile cycle of synthesis and breakdown. The hypothesis behind this cyclical metabolism is that it maintains the mitochondria in a metabolically readied condition for an immediate response to secondary challenge. Indeed, T cells with dysfunctional mitochondria are capable of mounting a primary response but are unable to clear reinfection[145].

1.5.4 Molecular regulation of T cell metabolism

Several signalling events take place downstream of antigen recognition and co-stimulation to guide metabolic reprogramming in T cells (Fig. 1.5 and Fig. 1.6). Mammalian target of rapamycin (mTOR) is a metabolic sensor and regulator, and critical in Teff development[154]. mTOR integrates external metabolite availability with stress, hormone and cytokine signals to regulate a diverse array of physiological functions, including metabolic adaptations to promote anabolic growth and proliferation[155]. In T cells, mTOR signalling is initiated by the PI3K-Akt pathway in response to TCR and CD28 ligation[141,156,157]. However, depending on the cytokine and nutrient environment, mTOR may be positively or negatively regulated to tailor metabolic reprogramming during T cell activation (Fig. 1.6).

mTOR is the central scaffold unit for two signalling complexes, mTORC1 and mTORC2, that signal through distinct adaptor proteins[155]. Activation of mTORC1 directly promotes protein translation, and initiates the transcriptional activity of HIF1α and c-myc, that directly bind and regulate metabolic genes for

glutaminolysis and glycolysis[140,158,159]. Activation of SREBPs, which control lipid biosynthesis, is also regulated by mTORC1[158]. In contrast to mTORC1, little is known about the downstream consequences of mTORC2 activity, though it may have important implications for cytoskeletal rearrangement and migration[160,161]. Its primary target for phosphorylation is Akt, which has many roles in supporting cell survival and proliferation, in part by phosphorylating mTORC1[155]. (Fig. 1.6) Overall, mTOR signalling is a strong promoter of biosynthetic pathways through the direct activation of transcription factors that target metabolic genes.

Signalling through mTOR is negatively regulated by activation of AMP-activated protein kinase (AMPK)[137] (Fig. 1.6). AMPK is activated by high intracellular levels of AMP and ADP in comparison to ATP, which is an indicator of metabolic stress. In a nutrient-limiting environment, it is detrimental for a cell to deplete ATP stores by continuing to use anabolic pathways for biosynthesis, when they can be used instead for distinct processes to keep the cell alive. Therefore, once available ATP levels drop below a certain threshold, AMPK inhibits anabolic metabolism by blocking mTOR activation. AMPK further prioritizes cell survival by promoting the breakdown of cellular organelles through the process of autophagy, increasing metabolite availability that can sustain ATP generation[162].

Activation of mTOR is essential for T cell activation. Deleting mTOR in T cells renders them unable to proliferate and become Teff[156]. This is also phenocopied by deleting many of the downstream targets of mTOR. T cells deficient in c-myc

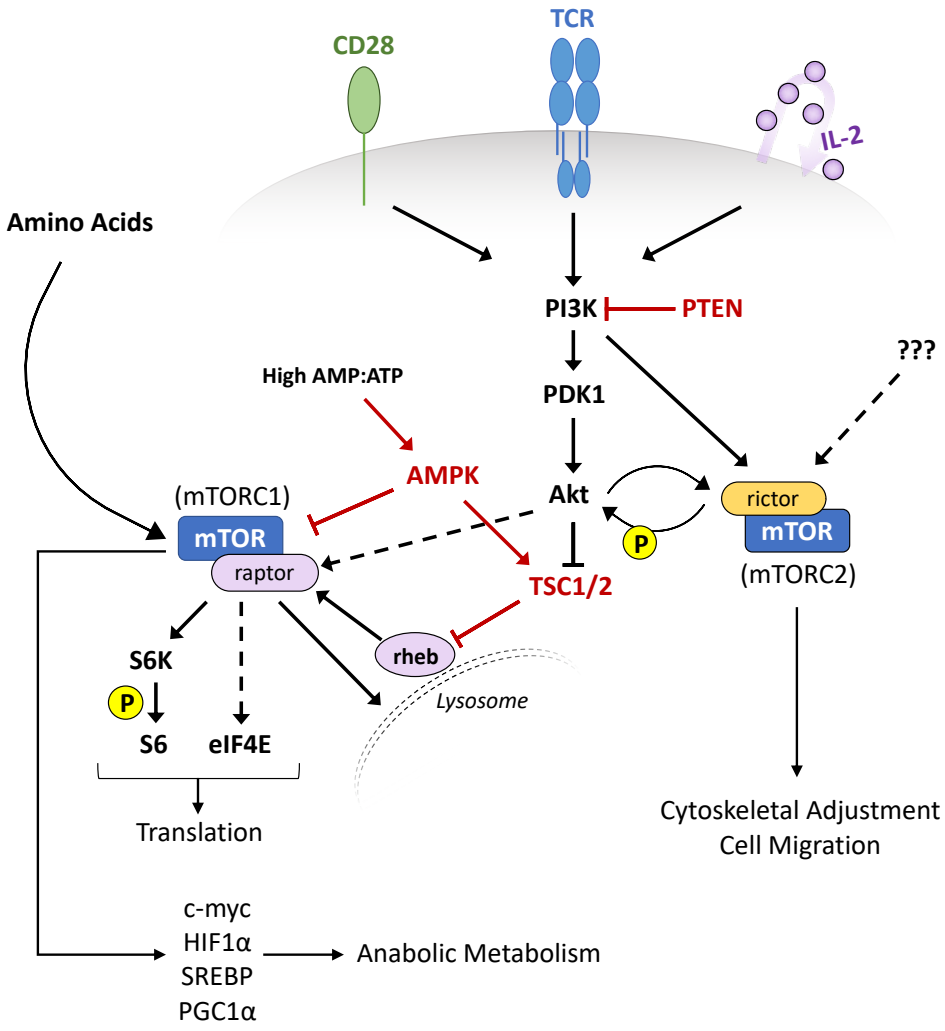

Figure 1.6 | Molecular regulation of T cell metabolism during activation

The PI3K-Akt-mTOR pathway is central to metabolic reprogramming. PI3K is recruited and activated following CD3 and CD28 ligation and activates mTORC2 to phosphorylate Akt. Akt promotes mTORC1 signalling through the deregulation of rheb. Rheb activates mTORC1 at the lysosome in response to increased amino acid levels. Anabolic metabolism, as well as protein translation, is largely driven by mTORC1 in T cells. The metabolic consequences of mTORC2 activation are less-well known. mTOR signalling can be tailored by additional signals, including cytokines, such as IL-2, which promotes PI3K signalling. Activation of mTORC1 or mTORC2 has distinct outcomes during T cell polarisation. Adapted from Saxton and Sabatini [155], and Pollizzi and Powell [154].

have a comparable proliferative defect to those deficient in mTOR, however cells lacking HIF1α are capable of proliferation similar to wild-type $CD4^+$ T cells, suggesting c-myc may be more important than HIF1α as an mTOR target for dictating T cell metabolism and activation[140]. T cells lacking SREBP similarly display impeded proliferation and blasting following CD3 and CD28 stimulation[163]. Conversely, cells without AMPK have the opposite phenotype to cells deficient in mTOR or downstream targets of mTOR, in that they are less restricted in becoming cytokine producing Teff. However, deleting AMPK removes a sensor that allows cells to metabolically respond to nutrient stress, thus AMPK-KO cells have a marked decrease in survival[137].

Peroxisome proliferator activated receptors (PPARs) are additional metabolic regulators, that have near complete transcriptional control of fatty acid metabolism[164]. This family of nuclear receptors are activated by endogenous lipid-based ligands which trigger their interaction with retinoid X receptors (RXRs), required partners for DNA recognition and binding[165]. Three isoforms exist, PPARα, PPARγ and PPARβ/δ, and each have varying functions that also differ for each isoform from tissue to tissue. In most tissues, PPARα and PPARβ/δ induce FAO, whereas PPARγ promotes FA storage and lipogenesis, particularly in adipocytes[165]. Interestingly, in immune cells PPARγ instead appears to also strongly promote FAO[166]. The transcriptional activity of all three PPARs can also be regulated by PGC1α (peroxisome proliferator-activated receptor-γ coactivator-

1α)[167]. PGC1α further stimulates mitochondrial biogenesis, and is therefore an important promoter of fatty acid metabolism, and mitochondrial OXPHOS[167].

PPARs have not been extensively studied in T cells, particularly in respect to metabolism, but the literary consensus is that PPAR activation negatively regulates Teff activation[168]. Treating naïve T cells with PPARγ agonists impaired CD3-induced proliferation and IL-2 production[169]. Furthermore, $CD4^+$ PPARγ-null mice display earlier onset and more severe symptoms of inflammatory diseases, including inflammatory bowel disease (IBD) and experimental autoimmune encephalomyelitis (EAE)[170–172]. PPARα and PPARβ/δ deficiency in the $CD4^+$ compartment also leads to exacerbated, T-cell dependent EAE severity in mice[173,174]. The role of PGC1α in T cells similarly still remains relatively unexplored. In the context of CD8-dependent anti-tumour or anti-viral immunity, PGC1α promotes a more adept cytotoxic T cell response due to enhanced mitochondrial metabolism, but not necessarily due to FAO[151,152].

1.5.5 Metabolic divergence in $CD4^+$ T cell subsets

The importance of metabolic reprogramming in T cells extends beyond just supporting activation. The balanced activity of metabolic pathways is intricately linked to subset specific differentiation and function of $CD4^+$ T cells[2,121,175]. A landmark paper by Michalek *et al* demonstrated that, *in vitro,* different T helper populations adopt different rates of aerobic glycolysis and FAO[176]. Differences were most pronounced when comparing Treg to Teff subsets. While Teff became

highly glycolytic, Tregs maintained a low rate of glycolysis but high OXPHOS, that was dependent on FAO. Further research elucidated that metabolism is central to the balance between Treg and Th17 polarization, both of which require TGF-β signalling for polarization[143,177–179]. The importance of Tregs in controlling disease, and their clearly divergent metabolic properties, has made Treg metabolism a topical area of research, however metabolic regulation and the requirements between different Teff subsets are more nuanced and still require much investigation.

Although distinct from that of Teff, Treg metabolism is still highly contextual[121]. Initial studies suggested Treg metabolism was directly opposite to that of Teff. Instead of depending on mTOR, Tregs required AMPK[176]. Teff needed glycolysis and FAS, but Tregs relied on FAO and low glycolysis[143,176,177]. In accordance with this notion, Foxp3 can directly promote the transcription of mitochondrial genes and has been shown to directly repress c-myc expression[180,181]. Treg are accordingly able to metabolically adjust for survival in a low-glucose environment[181]. Conversely, genetic models that force constitutively high glycolysis, such as HIF1α stabilization, negatively regulate Treg differentiation and suppressive capacity[182,183]. However, some level of glycolysis may be necessary for Treg function, as one group has reported that human *ex vivo* Tregs are highly glycolytic and require glycolysis for suppressive function[184,185]. Others, however, have proposed a model in which Tregs undergo a metabolic switch to transition

between either proliferative, or suppressive states[186]. Altogether, metabolic flexibility may be an inherent trait of Tregs that may be lacking in Teff[187].

While all Teff subsets are understood to be more highly glycolytic than Tregs, the initial study from Michalek *et al* reported that Th1, Th17 and Th2 cells exhibit different rates of glycolysis. These differences have been supported by multiple subsequent publications[176,177,188]. However, the functional meaning of this observation is still being explored. The first indication that differentiation to different $CD4^+$ T cell subsets had distinct metabolic requirements came from deleting different components of the mTOR complexes. Although mTOR is needed for all Teff development, the targeted deletion of rheb, an activating subunit of mTORC1, had a significant detrimental effect on Th1 and Th17, but not Th2 differentiation[156,189]. Conversely, Th2 polarization was significantly impaired by deficient mTORC2 activation, achieved through deletion of rictor, an essential component of the mTORC2 complex, or through knockout of the mTORC2-specific signal transducer rhoA[189–191]. It was later found that, despite rheb having no effect on Th2 cells, the signalling component of mTORC1, raptor, was critically required for Th2 differentiation[192]. Both mTORC1 and mTORC2 additionally have different roles in Tfh cell responses, but both are required for successful GC formation[193,194]. Altogether, therefore, tailoring the strength of mTOR signalling through the distinct complexes has large implications for the outcome of T cell activation.

Given the indispensable role of mTOR signalling in re-programming metabolism, altered mTOR signalling would be predicted to correlate with distinct metabolic requirements between different $CD4^+$ T cell subsets. Accordingly, several mechanistic examples of how metabolism is regulated, and contributes to T cell function in particular subsets, have been identified. In Th1 cells, glycolysis has a dual role in promoting IFNγ expression. The enzyme GAPDH carries out the sixth stage of glycolysis and directly inhibits IFNγ translation when its conventional substrate is unavailable, by binding 3' untranslated regions of *ifng* transcripts and sequestering it from ribosomes[146]. Glycolysis also provides a pool of acetyl-CoA which can be used to epigenetically regulate the *ifng* gene locus by histone acetylation[195,196]. GAPDH is also important for Th17 function, however increased glycolysis, specifically the breakdown of pyruvate to lactate, is key in minimizing ROS production that negatively regulates Th17 differentiation[124,197,198]. Recent studies have indicated that metabolic perturbation by IL-17 driven *raptor* deletion can repress the trans-differentiation of Th17 cells into more pathogenic Th1-like cells, suggesting Th1 cells may have a higher glycolytic threshold for cytokine production than that of Th17 cells[199].

Another dichotomous relationship has been described between Th1 and Th17 cells for glutamine metabolism. Glutamine-depletion, or removing the glutamine transporter ASCT2, inhibits differentiation of either cell type, indicating both rely on glutamine for optimal activation and cytokine production[135,200]. However, inhibiting glutaminolysis favours Th1 and diminishes Th17 differentiation, although

Th1 cells quickly adopt an exhausted phenotype when glutaminolysis is blocked[201]. The deviation between populations was attributed to a combination of increased ROS production and alterations in histone modifications regulating chromatin accessibility. Interestingly, Th2 cells are able to operate normally in the absence of glutamine or its transporter ASCT2[135,200].

Overall, with respect to core metabolism, how pathways contribute to specific Th function is likely highly context dependent, relying on external signals and nutrient availability. Using transgenic models, there is an apparent pattern that increased glycolysis favours Teff differentiation over Treg induction. However, a major gap still exists in the field pertaining to the *in vivo* behaviour of different Teff subsets. The characterization of T cell metabolism using physiologically relevant models will be critical in understanding the needs of particular immune cell populations, and what pathways can be targeted in situations of disease.

1.5.6 T cell metabolism in disease

A key motivation to understand how metabolism affects T cell function is for the potential application to disease management. It is evident there is a “sweet spot” in metabolism in T cells that ensures an appropriate immune response (Fig. 1.7). Activated T cells that are hypo-metabolic are unable to clear infection, or tumours, yet hyper-metabolic T cells may be an underpinning cause of autoimmunity[202]. Manipulating metabolism, particularly in T cells, is therefore a promising clinical target.

Exhausted T cells are T cells that have been activated through canonical TCR signalling but are functionally impaired due to environmental circumstances and/or repetitive stimulation by cognate antigen. Exhausted T cells are frequently observed in contexts of chronic viral infection, alloantigen reactions, and in cells under the influence of the tumour microenvironment. A characteristic feature of exhausted T cells in these scenarios is reduced glycolysis and dysfunctional mitochondrial metabolism compared to Teff[151–153]. In LCMV infection, $CD8^+$ T cells during an acute infection have enhanced glucose uptake, glycolysis and mitochondrial respiration, relative to those from a chronic infection. Restoring metabolic fitness through overexpression of PGC1α was able to restore T cell function[151]. Interestingly, in cancer, glucose competition with tumour cells is enough to restrain anti-tumour activity by T cells, such that restoring glucose concentrations within tumours is sufficient to revitalize T cell proliferation and cytokine expression[203]. Metabolism is now being taken into consideration for the design of chimeric antigen receptor T cell therapy, in order to improve long-lasting T cell survival and function within the tumour microenvironment[204,205].

Unregulated metabolic activity in T cells, on the other hand, is positively associated with inflammatory disease in murine models and human patients. $CD4^+$ T cells isolated from asthmatics had a higher propensity to use glycolysis when stimulated *ex vivo* and produced more Th2 cytokines compared to those from healthy controls. Th2 function was normalized by diverting glucose to towards the TCA cycle instead of lactate production[206]. Similarly, pathogenic alloreactive T cells

have a globally overactive metabolism, including aerobic glycolysis, glutaminolysis and FAO, following transplantation[207–209]. In EAE, a pathogenic subset of Th17 cells been characterized by increased anabolic activity[199]. This study showed that specific IL-17 driven deletion of *raptor* maintains IL-17 production in T cells, but results in a less pathogenic Th17 phenotype, and therefore reduced the severity of disease. Pathogenic Th17 cells were also recently described to have a dysregulated OXPHOS and lipid metabolism, where the latter provided ligands for the direct activation of RORγt[210,211]. Lastly, activated T cells in a murine model of systemic lupus erythematosus (SLE) were found to have high levels of glycolysis and OXPHOS that, when normalized, mitigated disease[212]. A similar metabolic dysfunction was seen in T cells from human lupus patients in comparison to those from healthy controls. Therefore, understanding immunometabolism in the context of T cell biology is a worthwhile pursuit for the advancement of therapies for a spectrum of disease.

1.6 Programmed cell death protein 1 (PD-1)

T cell activation can be tailored by several additional signals in addition to the TCR and CD28 co-stimulation. Multiple other co-stimulatory, and co-inhibitory, receptors can be expressed by T cells, which have different functional consequences as a result of ligation and subsequent signalling. PD-1 is a co-inhibitory receptor that has been well-characterized as a negative regulator of T

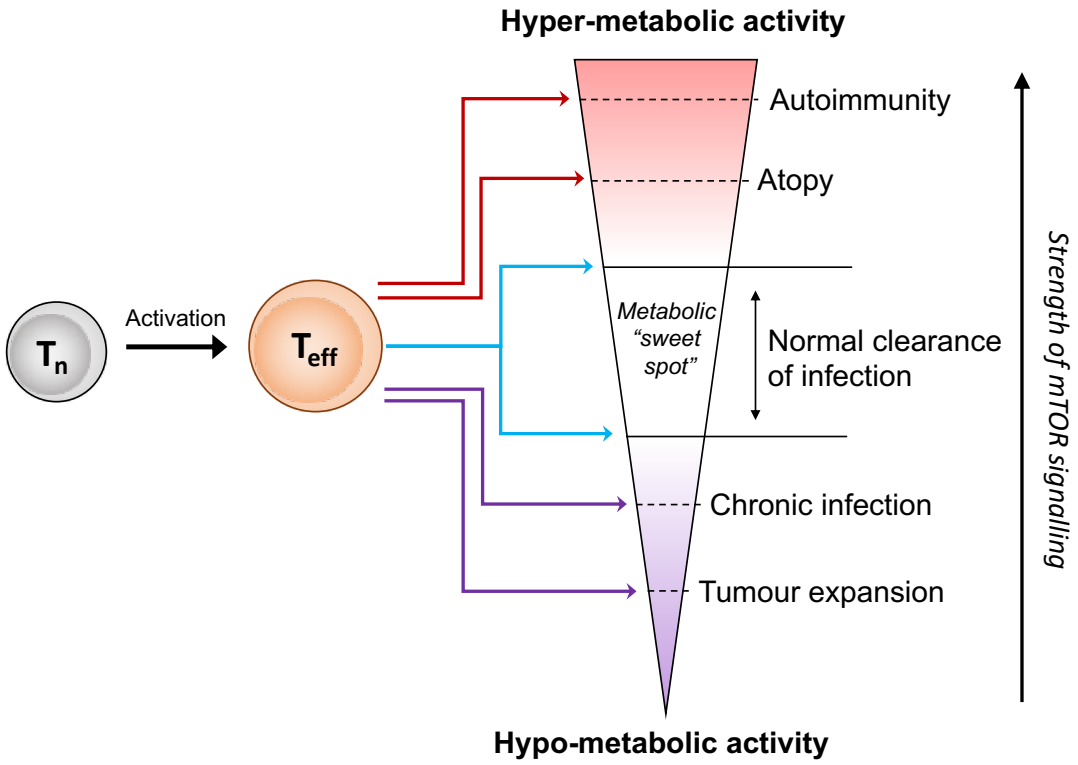

Figure 1.7 | T cell metabolism controls disease

T cell metabolism is observed to be dysregulated in a spectrum of human disease patients and experimental models. Overactive metabolism in T cells drives autoimmune reactions, which can be reverted through metabolic intervention. Insufficient metabolic reprogramming during T cell activation impairs immunity to infection and cancer. The metabolic activity of T cells is directly correlated to the strength of mTOR signalling. Adapted from Bantug *et al* [202].

cell responses[213]. In particular, it is strongly associated with establishing the functional exhaustion of $CD8^+$ T cells[214]. The role of PD-1 in driving $CD4^+$ T cell exhaustion has also been demonstrated, although potential subset specific consequences of PD-1 signalling have not been fully investigated[214].

1.6.1 Regulation of the T cell response by PD-1

Early in the T cell response, PD-1 serves as a marker of activation. Naïve T cells maintain low basal expression of PD-1, which may play a key role in immune tolerance[213,214]. Antigenic challenge leads to a transient upregulation of PD-1 expression, which then decreases in parallel to antigen clearance. However, persistent antigen-exposure causes T cells to maintain elevated levels of surface PD-1. It is thought that the major consequences of PD-1 ligation on T cells is inhibition of signals immediately downstream of the TCR and CD28[214,215]. Upon ligand binding, the cytoplasmic domain of PD-1 recruits inhibitory phosphatases that antagonize the phosphorylation cascade sustaining T cell activation[215,216]. PD-1 also interferes with the PI3K-Akt axis to restrain T cell activity[217,218].

Two ligands have been identified that bind PD-1 to induce signalling: PD-1 ligand 1 (PD-L1) and PD-L2[213]. Expression of these ligands is widely distributed across immune and non-immune cell-types, primarily within non-lymphoid tissues[219]. Hence, the major role of PD-1 is believed to be limiting T cell responses locally within the periphery. Although both ligands provide inhibitory signals via PD-1, their different roles in regulating T cell function has not been established[214]. PD-

L1 is more ubiquitously expressed than PD-L2, with PD-L2 expression being restricted mainly to DCs and macrophages. The two ligands are also differentially regulated by Th1 and Th2 cytokines, and alternatively expressed by different subsets of macrophages and DCs[220,221]. Therefore, both PD-L1 and PD-L2 may have distinct regulatory properties depending on the type of immune response.

Recent studies now suggest that PD-1 may have subset specific roles in promoting or restricting $CD4^+$ T cell responses. During chronic toxoplasmosis, as well as in solid tumours, PD-$1^{Hi}CD4^+$ T cells have limited effector function, characterized by reduced IFNγ expression relative to cells with intermediate or low PD-1 expression[152,222]. Furthermore, anti-PD-1 administration restores anti-tumour activity[152]. PD-1 similarly has a negative regulatory role on Tfh cells, as the absence of PD-L1 leads to exuberant Tfh expansion in draining lymph nodes[223]. In contrast, PD-1 signalling promotes peripheral Treg expansion, which may be another indirect role in which PD-1 dampens Teff responses[217]. PD-1 signalling has also been attributed to the induction of a TGF-β expressing subset of Th17 cells that drive pulmonary fibrosis in mice and humans, as well as the terminal differentiation of lung Th2 cells during helminth infection[83,224].

1.6.2 Metabolic consequences of PD-1 signalling

Exhausted T cells are metabolically distinct from fully functional Teff, and the metabolic dysfunction described in exhausted T cells has been attributed, in part, to signalling downstream of PD-1. *In vitro* ligation of PD-1 with soluble PD-L1

during T cell activation restricts glucose metabolism, consistent with the ability of PD-1 to suppress PI3K signalling[125,218]. PD-1 similarly causes a reduction in T cell OXPHOS[125,225]. Despite this, T cells activated in the presence of the ligand PD-L1 increase FAO[125]. Therefore, PD-1 induces a metabolic "switch" from glycolysis to FAO, rather than broadly suppressing Teff metabolism altogether. Interestingly, the metabolic traits associated with PD-1 signalling match those defined for Tregs, and may provide a rationale for the positive role of PD-L1 in Treg differentiation[176,217].

Genetically ablating PD-1 leads to the functional restoration of chronically stimulated T cells[151,153]. Indeed, in the absence of PD-1, T cells maintain a metabolically active phenotype, including increased glucose uptake and mitochondrial OXPHOS[151]. Mechanistically, PD-1 appears to be a negative regulator of PGC1α, and hence prevents mitochondrial biogenesis. PD-1 signalling causes the accumulation of large but depolarized and dysfunctional mitochondria, that can be prevented by the overexpression of PGC1α[151,152,226]. Similar observations of mitochondrial morphology have been made in T cells activated without co-stimulation, in keeping with the opposing actions of PD-1 and CD28[145]. Importantly, the efficiency of therapeutic PD-1 blockade on restoring T cell cytotoxicity against cancer is dependent on the tumour microenvironment[227]. Often, the concentrated mass of highly metabolic tumour cells creates a nutrient-deplete and hypoxic niche, which is hypothesized to be a major barrier to effective immune responses. Indeed, it has recently been demonstrated that altering the

oxidative metabolism of cancer cells increases the effectiveness of anti-PD-1 therapy[228,229]. These studies indicate that the metabolic constraint imposed by PD-1 is critical to its functional inhibition of T cells. Furthermore, observations of the impact of metabolism and nutrient availability on the success of anti-PD-1 therapy emphasizes the importance of immuno-metabolic considerations in conceiving new therapeutic innovations

1.7 Research aims and rationale

While metabolism has become a popular avenue for immunologists, studies of T cell metabolism have largely neglected Th2 cells. The central aim of this study is to determine the metabolic phenotype of Th2 cells and how metabolism is regulated *in vivo* during the anti-helminth immune response. As targeting immunometabolism has been highlighted as a promising therapeutic strategy in auto-immunity, cancer and chronic viral infection, similar principals may be applicable to Th2 associated disease, including the design of anti-helminth vaccinations. Furthermore, since the discovery of Th2 cells in the 1980's, the signals needed for *in vivo* Th2 differentiation still represent a major gap in our knowledge of $CD4^+$ T cell biology. Hence, understanding the metabolic characteristics and requirements of Th2 cells may yield valuable insights into how Th2 cells develop during infection.

My first aim in this study was to characterize the importance of glycolysis in Th2 function, *in vivo*, during helminth infection. I hypothesized that, similar to other Teff subsets, and to what has been reported *in vitro*, that helminth-activated Th2 cells would be highly glycolytic[176].

The second aim of this study was to assess the ability of helminths to suppress Th2 cell metabolism. Helminths impose a highly suppressive environment that impedes the host immune response designed to expel them[52,67]. In answering my first aim, I found that Th2 cells maintained an unusual, glycolytically-low metabolism. I hypothesized that helminth-mediated immune suppression contributed to dampening Th2 glycolysis.

It has been demonstrated that Th2 differentiation is a multi-step process in which, after lymph node priming, complete effector differentiation is achieved after migration to the affected peripheral tissue[41,43]. Therefore, for the last aim of this study I sought to determine if metabolic differences are apparent between primed Th2 cells in the lymph node and fully functional Th2 cells in the effector site. Given that my first two aims revealed surprisingly low glycolysis in Th2 cells isolated from the lymph node, I predicted that in the peripheral tissue, Th2 cells would increase glycolytic metabolism to support enhanced effector function.

Overall, all three aims were designed to contribute to the current understanding of metabolism, activation and regulation of Th2 cells during *in vivo* responses, in the context of infection.

Chapter 2: Materials and Methods

2.1 Mice

Wild-type C57BL/6 mice were obtained in Canada from Jackson Laboratories. B6.4get and Great mice were ordered initially from Jackson Laboratories then bred in-house under specific pathogen-free conditions at the University of British Columbia (Chapter 3, Fig. 4.1 and 4.5A). In the United Kingdom C57BL/6 mice were obtained from Envigo. B6.4get mice were generously provided by Dr. John Grainger at the University of Manchester and bred in-house at the University of Glasgow. Mice were maintained in IVC cages in conventional facilities (Chapters 4 and 5). All animal work was conducted in accordance with the guidelines set by the University of British Columbia Animal Care Committee and the Canadian Council of Animal Care, or with the approval of the University of Glasgow Animal Welfare and Ethics Review Board and done under licensing issued by the UK Home Office. Mice were infected between 6-12 weeks of age, sex and aged matched for each independent experiment.

2.2 Pathogen cultivation and infection

To maintain larval stocks of *H. polygyrus*, male mice of at least 12 weeks of age on a C57BL/6 background were infected with 400 L3 larvae for 3-4 weeks. Faeces from the colon and large intestine were collected and spread evenly in a thin layer on 6 pieces of layered 125mm Whatman filter paper made damp with sterile distilled water, in a 150mm dish. Faecal plates were kept surrounded by damp

paper towel in a plastic tub at room temperature in the dark for 10-12 days. Free larvae were collected by washing the bottom 2 layers of Whatman filters with distilled water. Larvae were washed several times with water and kept at 4°C until needed for infection. Experimental infections were done using 200 L3 infective larvae and administered in 0.2 ml by oral gavage. *H. polygyrus* larvae used in the UK were generously provided by Prof. Rick Maizels.

For *Toxoplasma gondii*, mice were experimentally infected with *T. gondii* strain ME49 10 cysts of by oral gavage. Cysts were obtained on the day of infection from the brains of chronically infected C57BL/6 mice previously infected with 40 cysts. Source mice were infected for a minimum of 3 weeks prior to use. Brain tissue was harvested into Hanks buffered saline solution (HBSS) (ThermoFisher), manually homogenized in a 15ml tube and filtered through a 100µm strainer for counting. Homogenized tissue was diluted appropriately with phosphate buffered saline (PBS) (ThermoFisher). *T. gondii* infections for Fig. 4.5B and C were done by Prof. Craig Roberts at the University of Strathclyde, Glasgow, UK.

Influenza A virus PR8 strain was administered intranasally to mice anesthetized with isoflurane. Mice were infected with 5 pfu of virus in 25µl. *Nippstrongylus brasiliensis* infection was done by sub-cutaneous injection of 250 L3 larvae. *N. brasiliensis* larvae were cultivated and obtained from Prof. Rick Maizels' group at the University of Glasgow.

2.3 House-dust mite challenge

HDM extract was obtained from GREER Laboratories. Mice were administered either 25µl of 25µg of HDM or 25µl of PBS for 4 consecutive days intranasally under anaesthesia. Sensitized mice were challenged 10 days later with 25µg of HDM extract for another 4 days while control mice received additional doses of PBS. Mice were culled 2 days after the final challenge dose.

2.4 Antibodies and reagents

Staining for flow cytometry was done using the following anti-mouse monoclonal antibodies: CD4 (RM4-5) conjugated to e450, APC or APC-Cy7 (BioLegend); CD44 (IM7) conjugated to APC or PE-Cy7 (BioLegend); TCRβ (H57-597) conjugated to PerCP-Cy5.5 (Biolegend); ST2 (DIH9), conjugated to BV421 or PE (BioLegend); PD-1 (29F.1A12) conjugated to PE or BV711 (BioLegend); CXCR5 (SPRCL5) conjugated to biotin (ThermoFisher); IL-5 (TRFK5) conjugated to PE (BioLegend); IL-13 (eBio13A) conjugated to e660 (ThermoFisher); IFNγ (XMG1.2) conjugated to e450 (ThermoFisher); Foxp3 (FJK-16s) conjugated to e450 or APC (ThermoFisher); GATA3 (TWAJ) conjugated to PE (ThermoFisher); phospho-S473 Akt (M89-61) conjugated to PE (BD Bioscience); phospho-S235/236 S6 (N7-548) conjugated to Alexa Fluor 647 (BD Bioscience). Streptavidin conjugated to APC was used to detect biotin-CXCR5 (ThermoFisher). Separation of dead cells was achieved using e506 or e780 fixable eViability dye (ThermoFisher).

2NBDG and BODIPY FL C16 were purchased lyophilized from ThermoFisher. 2NBDG (2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose) was reconstituted in PBS. BODIPY FL C16 (4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-*s*-Indacene-3-Hexadecanoic Acid) was reconstituted by shaking for 1 hour in 1% fetal bovine serum (FBS) in PBS.

Cells were cultured in complete RPMI (cRPMI) media: RPMI 1640, 10% FBS, 1 mM Glutamax, 1 mM penicillin-streptomycin, 1mM non-essential amino acids, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol (ThermoFisher). 2-deoxy-d-glucose (2DG) (Sigma-Aldrich) was reconstituted in base DMEM (Aligent). Glucose-free cultures were done with completely supplemented glucose-free RPMI 1640 and appropriately diluted sterile glucose solution (Sigma-Aldrich). *H. polygyrus* excretion and secretions (HES) was generously provided by Prof. Rick Maizels, purified according to published protocol[230]. Surface staining was done in FACS buffer: PBS pH 7.4, 2% FBS, and 2 mM EDTA (ThermoFisher).

2.5 *In vitro* CD4$^+$ T cell polarization

For *in vitro* cultures, CD4$^+$ T cells were isolated from naïve splenocytes using the EasySep mouse CD4$^+$ T cell enrichment kit (StemCell Technologies) and plated at 3x10^5 cells per well in a flat bottom 96 well plate. Stimulation was done using 1μg/ml plate-bound αCD3 and 1 ug/ml soluble αCD28 (eBioscience) in polarizing conditions; Th1: 10 ng/ml IL-12 (ThermoFisher), 10 ng/ml IL-2 (ThermoFisher), 1ug/ml αIL-4 (UBC Ablab/BioLegend); or Th2: 40 ng/ml IL-4 (ThermoFisher), 10

ng/ml IL-2 (ThermoFisher), 1 µg/ml αIFNγ (UBC Ablab/BioLegend). Where stated, cells were treated with 0.5 mM 2DG, 5 µg/ml of HES, 20 ng/ml of TGF-β (ThermoFisher), or 20 ng/ml of *Hp*-TGM. Activated cells were sorted or analysed 4 days after stimulation.

2.6 *Ex vivo* CD4$^+$ isolation and restimulation

Lymph nodes from infected mice were directly crushed into a single-cell suspension through a 70µm strainer in cRPMI. To obtain lung T cells, tissue was first digested before being filtered through a 70µm strainer (see 2.7). Single-cell suspensions were enriched for CD4$^+$ cells using either the EasySep mouse CD4$^+$ T cell enrichment kit (StemCell Technologies), or MojoSort^{TM} CD4 T cell isolation kit (BioLegend), before being FACS-sorted. *Ex vivo* sorted cells were plated in 96 well plates as indicated: αCD3, 1 µg/ml plate-bound; 2DG, 0.5 mM (Sigma-Aldrich); IL-33, 20 ng/ml (BioLegend); PD-L1-Fc, 5 µg/ml plate-bound (R&D Systems).

2.7 Lung harvest and digestion

Mice were euthanized by overdose of intraperitoneally injected anaesthetic and lungs were perfused with ice-cold PBS before being removed. Lungs were transferred to 1 ml of digest buffer and cut into 1 mm pieces. Digest buffer was made by reconstituting 5mg of Liberase TL (Roche) in 2ml of water and using it at a 1/6.5 dilution (~2 U/ml) in HBSS containing Mg^{2+} and Ca^{2+} (ThermoFisher), and 50U/ml of DNAse I from bovine pancreas (Sigma-Aldrich). Cut lung pieces were

digested for 35 min at 37°C shaking at 185rpm. Digestion was halted by added 5ml of ice-cold cRPMI before crushing digested tissue through a 70µm strainer and washed through with 25 ml of cold cRPMI. Cells were spun down at 400xg for 5 minutes and resuspended in LCK buffer (ThermoFisher) for 3 min to lyse red blood cells, before being washed with an addition 25 ml of cRPMI. Cells were spun and resuspended appropriately for downstream analysis.

2.8 Flow cytometry and cell-sorting

In vitro cultures of purified $CD4^+$ cells were directly stained with fixable eViability dye (ThermoFisher) and antibodies against surface antigens in FACS buffer (1x PBS pH 7.4, 2% FCS, 2mM EDTA) for 30 minutes at 4°C. Tissue samples being directly analysed *ex vivo* were Fc-blocked by incubation with 5 µg/ml αCD16/32 (BioLegend) for 10 minutes prior to viability and surface staining.

For intracellular cytokine staining, cells were re-stimulated with 50 ng/ml phorbol-13-myristate-12-acetate (PMA) (Sigma-Aldrich) and 0.5 µg/ml ionomycin (Sigma-Aldrich) for 5 hours in the presence of 1x monensin (Thermo Fisher). Cells were surface fixed and permeabilized with BD Cytofix for 20 minutes and stained for intracellular cytokines either at 4°C overnight or for at least 1 hour at room temperature. Cells were washed with eBioscience Foxp3 perm/wash buffer (ThermoFisher) and resuspended in FACS buffer for analysis.

To maintain reporter expression, transcription factor staining was achieved by fixing cells with BD Cytofix for 1 hour at room temperature after surface staining in FACS buffer. Cells were then washed with eBioscience Foxp3 perm/wash buffer and stained overnight at 4°C, or at room temperature for a minimum of 2 hours. In Fig. 3.7 and Fig. 4.1, cells were first fixed using BD Cytofix with 0.01% Triton-X, then stained for GFP using polyclonal rabbit αGFP primary and α-rabbit secondary antibodies for 1 hour each, to recover GFP detection after secondary fixation with eBioscience Foxp3 fix/perm buffer for intranuclear staining of Foxp3 and Ki67, done for 30 minutes at room temperature.

Phosflow staining was done using Perm Buffer III (BD Biosciences) according to the manufacturer's protocol. *Ex vivo* phos-flow analysis was done by resuspending MLN single-cell suspensions directly in pre-warmed 1x fixation buffer. Cells analysed from *in vitro* cultures were transferred to 5ml tubes and allowed to rest for 2 hours before fixation. Where indicated *in vitro* cells were first re-stimulated with 50 ng/ml of PMA for 30 minutes.

Data for stained cells was acquired using an LSRII (BD Bioscience) in Vancouver and either a BD LSRII or BD Fortessa in Glasgow. Cell sorting was done using a BD Influx or BD Aria II. $CD4^+$ T cells were sorted into 2 ml of cRPMI using a 100µm nozzle. Analysis was done using FlowJo (TreeStar).

2.9 RNA isolation and quantitative-PCR

RNA was extracted from sorted cells using the RNeasy mini kit (Qiagen) or RNAqueous Micro Kit (Ambion). Concentrations of RNA were determined using a nanodrop 1000. 0.1-1.0 µg of RNA was converted to cDNA using the iScript cDNA synthesis kit (Bio Rad) or the High-Capacity RNA-to-cDNA kit (ThermoFisher). qPCR was done using either SsoFast EvaGreen supermix and a BioRad CFX96 Real Time System (Vancouver), or PowerUp SYBR Green master mix (ThermoFisher) using a QuantStudio 6 Flex Real-time PCR system (Applied Biosystems) (Glasgow). Target gene expression was normalized to the expression of the ribosomal protein S29 (*RPS29*) using the △Ct method. *HK2, ENO1, PKM2* and *LDHA* primer sequences were obtained from Shi *et al*, 2011 [177]; *PPARG* sequences from Nobs *et al,* 2017 [231]; *CD36* sequences from Matsusue *et al,* 2014 [232]; *CPT1A* sequences from Byersdorfer *et al*, 2013 [233]; *FABP4* and *FABP5* from Pan *et al*, 2017 [234]; *FASN, ACLY, ACACA* from Young *et al*, 2017 [235]. Primers were order from ThermoFisher.

HK2 Fwd 5'- TGA TCG CCT GCT TAT TCA CGG -3'
Rev 5'- AAC CGC CTA GAA ATC TCC AGA -3'

ENO1 Fwd 5'- TGC GTC CAC TGG CAT CTA C -3'
Rev 5'- CAG AGC AGG CGC AAT AGT TTT A -3'

PKM2 Fwd 5'- GCC GCC TGG ACA TTG ACT C -3'
Rev 5'- CCA TGA GAG AAA TTC AGC CGA G -3'

LDHA Fwd 5'- CAT TGT CAA GTA CAG TCC ACA CT -3'
Rev 5'- TTC CAA TTA CTC GGT TTT TGG GA -3'

ACLY Fwd 5'- GGT GAC TCC CGA CAC AGA CT -3'
Rev 5'- AAG CTT TCC TCG ACG TTT GA -3'

ACACA	Fwd 5'- TGG CTT CTC CAG CAG AAT TT -3'
	Rev 5'- AGA TCG CAT GCA TTT CAC TG -3'
FASN	Fwd 5'- AGT TGC CCG AGT CAG AGA A -3'
	Rev 5'- CGT CGA ACT TGG AGA GAT CC -3'
CD36	Fwd 5'- GAT GAC GTG GCA AAG AAC AG -3
	Rev 5'- TCC TCG GGG TCC TGA GTT AT -3'
FABP4	Fwd 5'- TTT CCT TCA AAC TGG GCG TG -3'
	Rev 5'- CAT TCC ACC ACC AGC TTG TC -3'
FABP5	Fwd 5'- AAC CGA GAG CAC AGT GAA G -3'
	Rev 5'- ACA CTC CAC GAT CAT CTT CC -3'
CPT1A	Fwd 5'- AGA TCA ATC GGA CCC TAG ACA C -3'
	Rev 5'- CAG CGA GTA GCG CAT AGT CA -3'
PPARG	Fwd 5'- GTG ATG GAA GAC CAC TCG CAT T -3'
	Rev 5'- CCA TGA GGG AGT TAG AAG GTT C -3'
GAPDH	Fwd 5'- GTG TTC CTA CCC CCA ATG TGT -3'
	Rev 5'- ATT GTC ATA CCA GGA AAT GAG CTT-3'
RPS29	Fwd 5'- ACG GTC TGA TCC GCA AAT AC -3'
	Rev 5'- CAT GAT CGG TTC CAC TTG GT -3'.

2.10 Seahorse metabolic flux assay

$2x10^5$ sorted *in vitro* or $4x10^5$ sorted *ex vivo* activated or naive cells were plated in glucose-free minimal DMEM (Aligent Technologies), incubated for 1 hour at 37°C, and analysed using a Seahorse XFe96 Bioanalyzer. ECAR and OCR were measured following injection of 10 mM glucose, 2 μM oligomycin, and 50 mM 2DG (Sigma-Aldrich). Results are shown normalized to cell number. Calculations were performed using the greatest values of individual wells after each injection.

2.11 *In vivo* injections

For 2-NBDG uptake, mice were injected intravenously with 100 µg of 2-NBDG diluted in sterile PBS 1 hour prior to sacrifice. To deplete regulatory T cells, 1 mg of αCD25 antibody (PC61.5.3, Bio X Cell) per mouse was injected intraperitoneally one day before infection. 1 mg of Rat IgG1 (Ablab) was used as an isotype control.

2.10 Metabolite uptake and mitochondrial staining

To determine 2NBDG and BODIPY FL C16 uptake single-cell suspensions from harvested tissues pooled and $CD4^+$ T cells were purified using the MojoSort $CD4^+$ isolation kit. A 96 well round-bottom plate was seeded with $2.5x10^5$ purified $CD4^+$ cells and rested in cRPMI for 30 minutes at 37°C. Cells were spun down and resuspended in 50 µM 2NBDG or 5 µM BODIPY FL C16 diluted in PBS and incubated at 37°C for 15 minutes before being quenched with ice-cold FACS buffer, washed twice, and stained for surface markers. To stain for mitochondria, single-cell suspensions were incubated for 20 minutes with 50 nM of MitoSpy Orange CMTMRos (BioLegend) and 50 nM of MitoTracker DeepRed FM (ThermoFisher) in RPMI containing no additives. Cells were washed twice with PBS before additional staining.

2.11 Statistical analysis

Statistics were assessed using GraphPad Prism version 8. For direct comparison between 2 groups, an unpaired students T test was used. For comparisons

between 3 or more groups, a one-way analysis of variance (ANOVA) was performed with Tukey's multiple comparison correction. In cases where groups had unequal standard deviations, unpaired T-tests were run with Welch's correction or Brown-Forsythe and Welch ANOVA was used with the Dunnett T3 correction for multiple comparisons. Data are presented as the mean with standard deviation. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$, ns = not significant.

Chapter 3: A distinct metabolic phenotype of Th2 cells *in vivo*

3.1 Introduction and rationale

T cell activation rapidly orchestrates metabolic changes within the cell[2,46,236]. TCR stimulation triggers concomitant increases in oxidative phosphorylation (OXPHOS) and aerobic glycolysis[141,142,146]. However, glycolysis has a proportionally greater increase, leading to a predominantly anabolic metabolism. This shift is thought to allow faster generation of ATP, to increase the pool of biosynthetic precursors, and to replenish redox-balancing intermediates in order to sustain the growth and proliferation associated with the T cell transition into an effector state[2,121]. Accordingly, ablating the ability of T cells to engage glycolysis leaves hosts susceptible to viral infection[151,237] and unrestrained tumour growth[203]. Conversely, dysregulated or excessive T cell glycolysis is a critical driver of pathology in several models of disease[202], including multiple sclerosis (EAE)[177,197], lupus[212], and GVHD [207,225].

Glycolysis is also important in regulating T cell function within disparate T helper cell populations[121]. Current dogma is that glycolysis is tightly paired to Th2 cell activation and function[121,175,202]. Naïve $CD4^+$ T cells polarized *in vitro* under Th2 conditions present with the highest glycolytic rate compared to cells cultured in Th1, Th17 and T_{reg} conditions[176,177]. Th2 cells have also been shown to highly express the glucose transporter Glut1[127], the primary transporter required for

glucose uptake in T cells, and to have the highest expression of Glut1 among other $CD4^+$ Th subsets[176].

Glycolysis is also vital for Th2 cytokine production *in vitro*. Pharmacological inhibition of glycolysis with low doses of the glucose analogue 2-deoxy-d-glucose (2DG) completely abrogates IL-4 production and IL-13 transcription by Th2 polarized cells, with no apparent detriment to activation or proliferation[191,192]. Mechanistically, it has been proposed *in vitro* that glucose restriction prevents Th2 differentiation by limiting expression of the IL-4 receptor alpha chain (IL-4Rα), the IL-2Rα, and the Th2 master transcription factor GATA3[192].

Impairing the ability of Th2 cells to engage glycolysis *in vivo* using a transgenic T cell knockout of the mammalian target of rapamycin (mTOR) also translated to reduced cytokine release, fewer tissue-infiltrating immune cells, and less severe pathology in murine models of asthma and allergy[192]. Interestingly, circulating T cells from asthmatic human patients also have a propensity to use greater glycolysis than those from healthy controls when stimulated *ex vivo*[206]. Normalizing glycolysis with dichloroacetic acid (DCA), a compound that redirects pyruvate toward the TCA cycle, normalizes cytokine output to control levels in $CD4^+$ T cells from these asthma patients.

Combined these data have been interpreted to mean that a high glycolytic rate is an inherent feature of Th2 cells, but this interpretation has been derived mainly

from *in vitro* models. The metabolic properties of Th2 cells have yet to be characterized directly from an *in vivo* source during an ongoing immune response. Importantly, T cell metabolism has also not yet been investigated in the context of helminth infection. Helminths are potent drivers of the Th2 response and as human pathogens are one of the most prevalent infectious agents in the world[52]. Targeting immunometabolism has been highlighted as a promising therapeutic strategy in auto-immunity[238], cancer[202] and chronic viral infection[239], and similar principles may be applicable to Th2-associated disease.

In this chapter, I aimed to determine the glycolytic phenotype of Th2 cells activated *in vivo,* during an ongoing type 2 immune response. Mice were infected with *Heligmosomoides polygyrus*, a model of soil-transmitted helminth infection in humans[68]. As a comparison, I used the protozoan parasite *Toxoplasma gondii* to generate and assess an enteric Th1 response. My hypothesis was that Th2 cells would show higher glycolysis than Th1 counterparts both *in vitro* and *in vivo*. I further hypothesized that this increase in glycolysis would be needed to support Th2 cytokine production.

3.2 Results

3.2.1 *In vitro* activated Th2 cells are highly glycolytic

I first compared glycolysis in activated Th1 and Th2 cells *in vitro*. Previous research has shown that *in vitro* activated Th2 cells are more glycolytic than other equivalently activated Th subsets[176,177]. A potential caveat of these studies was

that bulk CD4$^+$ T cell populations were analysed from polarized cultures without selecting for active, cytokine producing cells. In order to analyse a homogenous population of polarized and equivalently activated Th cells, I activated naïve CD4$^+$ T cells from either *il4*-eGFP (B6.4get)[240] or *ifng*-eYFP (Great) mice[29] in the presence of polarizing cytokines. On day 4 post-activation, reporter-positive cells were purified for analysis by FACS (Fig. 3.1A). Relative levels of glycolysis were first gauged using qPCR to assess the expression of key genes in the glycolysis pathway, including hexokinase 2 (HK2), enolase 1 (ENO1), pyruvate dehydrogenase M2 (PKM2) and lactate dehydrogenase A (LDHA) (Fig. 3.1B, C). Th1 and Th2 cells both highly up-regulated gene expression for glycolytic enzymes, relative to freshly isolated naïve CD4$^+$ controls. However, Th2 cells showed a non-significant but consistent trend for a greater increase in glycolytic gene expression compared to the increase from naïve cells observed for Th1 cells (Fig. 3.1C). This implies that activated, cytokine-producing Th2 cells may use more glycolysis than Th1 counterparts.

Changes in gene expression do not necessarily indicate enzymatic activity, and therefore I directly measured rates of cellular metabolism using a Seahorse extracellular flux assay. Specifically, isolated cytokine-expressing cells were subjected to the glycolysis stress-test, as outlined in Figure 3.2A, to determine their metabolic response to glucose. In a 96 well format, basal glycolysis is first measured following glucose starvation, and then after a subsequent injection of glucose. The rate of glycolysis is determined by the extracellular acidification rate

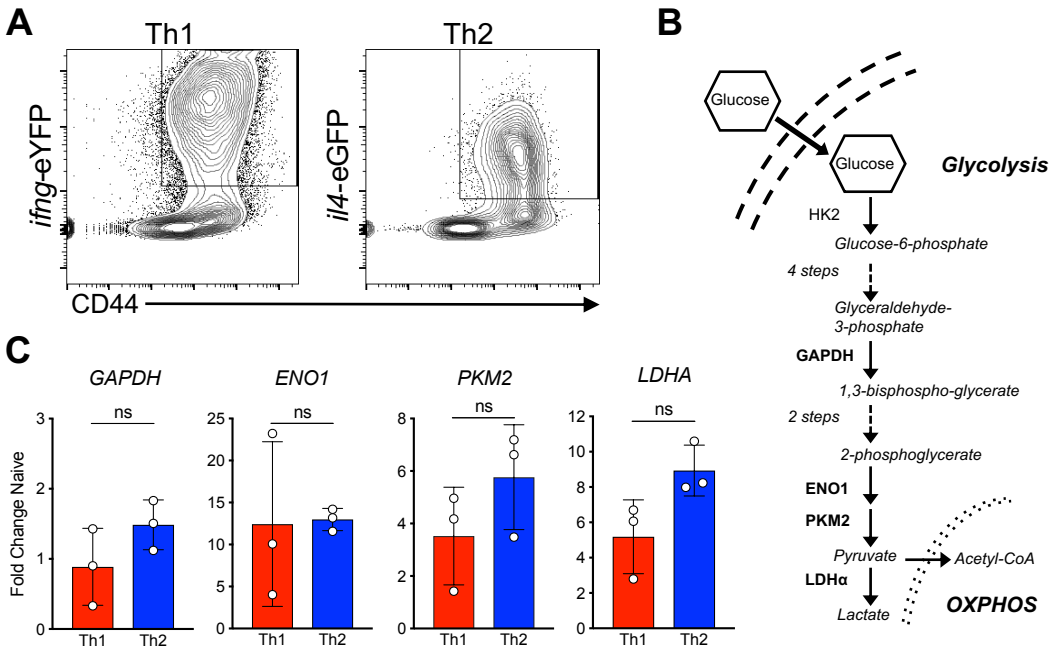

Figure 3.1 | Glycolytic gene expression of *in vitro* polarized Th1 and Th2 cells

Total CD4^{+} T cells were isolated from either *ifng*-eYFP (Great) or *il4*-eGFP (B6.4get) mice by negative selection and activated in Th1 or Th2 polarizing conditions, respectively, before cell-sorting and analysis. **A**, Representative reporter expression in polarised Th1 and Th2 for cell-sorting. **B**, Simplified overview of the glycolysis pathway highlighting enzymes analyzed for gene expression. **C**, Gene expression of glycolytic enzymes in sort-purified YFP^{+} Th1 and GFP^{+} Th2 relative to strain matched CD4^{+}CD44^{Lo} naïve splenocyte controls, determined by qPCR. Individual data points represent independent experiments.

(ECAR), as lactic acid is secreted by the cells as lactate and hydrogen ions. After glucose injection, ECAR was highly elevated in Th1 and Th2 cells, indicating that both cell types respond rapidly to glucose and are highly glycolytic (Fig. 3.2B, C). In contrast, freshly isolated naïve $CD4^+$ T cells had a low glycolytic rate when supplied with glucose. Compared to Th1 cells, Th2 cells had a small but still significantly higher rate of basal glycolysis (Fig. 3.2B, C).

To determine if cells are operating at their maximal glycolytic rate, ECAR was measured after the addition of oligomycin. Oligomycin inhibits the ATP synthase of the electron transport chain (ETC), forcing cells to compensate by increasing glycolysis to their maximum potential. In the presence of oligomycin, both Th1 and Th2 cells underwent only marginal increases in ECAR (Fig. 3.2B, C). The two groups also showed no difference in their glycolytic reserve, the relative difference between maximal and basal glycolysis. Therefore, both cell populations are operating near their full glycolytic potential, but Th2 cells maintain a slightly higher rate of glycolysis over that of Th1 cells.

During the Seahorse assay, the oxygen consumption rate (OCR), indicative of mitochondrial ETC activity, is simultaneously measured. Th2 cells also had a higher OCR, and therefore also had a higher propensity for OXPHOS compared to Th1 (Fig. 3.2D, E). This meant the ratio of OCR to ECAR was similar between Th1 and Th2 cell subsets, in that both have a dominantly glycolytic metabolism to a similar degree (Fig. 3.2F).

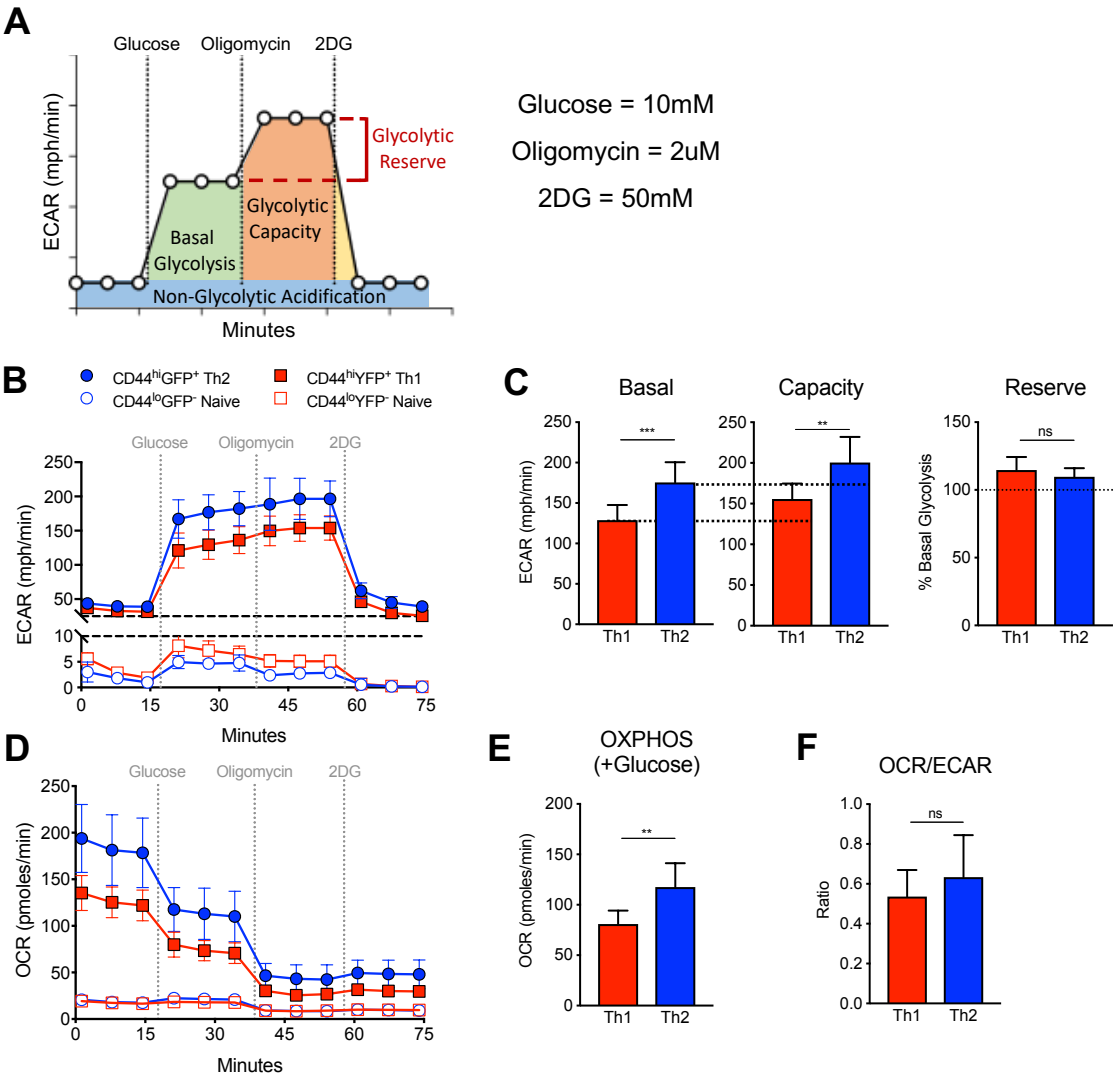

Figure 3.2 | Th1 and Th2 cells are highly glycolytic *in vitro*

Reporter-positive Th1 and Th2 cells polarized *in vitro* were sorted and analyzed using Seahorse extracellular flux. **A**, Overview of the glycolytic stress-test. **B**, Extracellular acidification rate (ECAR) of sorted Th1 and Th2 cells, as well as CD44^{lo} naïve controls. **C**, Bar chart representation of basal glycolysis, glycolytic capacity and glycolytic reserve. **D**, Matched oxygen consumption rate (OCR) and **E**, Graphed OCR following glucose injection. **F**, Measured OCR divided by ECAR following glucose injection and prior to oligomycin injection. Data shown pooled from 3 independent experiments. Graphed data represents the average of the highest measured values from each replicate.

Overall, consistent with previously published studies, these data show that Th2 cells activated *in vitro* display a higher glycolytic rate, as well as OXPHOS, than similarly activated Th1 cells, yet both subsets are highly and maximally glycolytic.

3.2.2 Th2 cells exhibit a low glycolytic rate *ex vivo*

To assess T cell glycolysis in a physiological setting, *in vivo* responses were compared by infecting B6.4get mice with *H. polygyrus* and Great mice with the protozoan parasite *T. gondii*. The subsequent Th2 and Th1 cells activated during infection were sort-purified from the mesenteric lymph nodes (MLN) of infected mice during the peak of the primary T cell responses, day 14 and day 7 respectively[241,242], according to *il4* and *ifng* expression (Fig. 3.3A, B). $CD4^+CD44^{Lo}$ cells from uninfected mice were sorted in parallel as naïve T cell controls. In contrast to the high glycolysis observed *in vitro*, Th2 cells from helminth infected mice showed strikingly low glycolytic gene expression. Relative to naïve $CD4^+$ cells, Th2 cells had no significant change in enolase (*ENO1*) or pyruvate kinase (*PKM2*) expression (Fig. 3.3C). *T. gondii* activated Th1 cells, however, highly expressed glycolytic genes, more closely resembling *in vitro* activated cells (Fig. 3.3C, compared to Fig. 3.1C). Significant increases in expression of the first and last enzymes of glycolysis, *HK2* and lactate dehydrogenase (*LDHA*) were observed in *H. polygyrus*-activated Th2 cells, but this expression was low compared to that of *T. gondii*-activated Th1 cells (Fig. 3.3C). Expression of *HK2* in Th1 cells was over tenfold greater than that of Th2 cells, and approximately 3-fold more for *LDHA*.

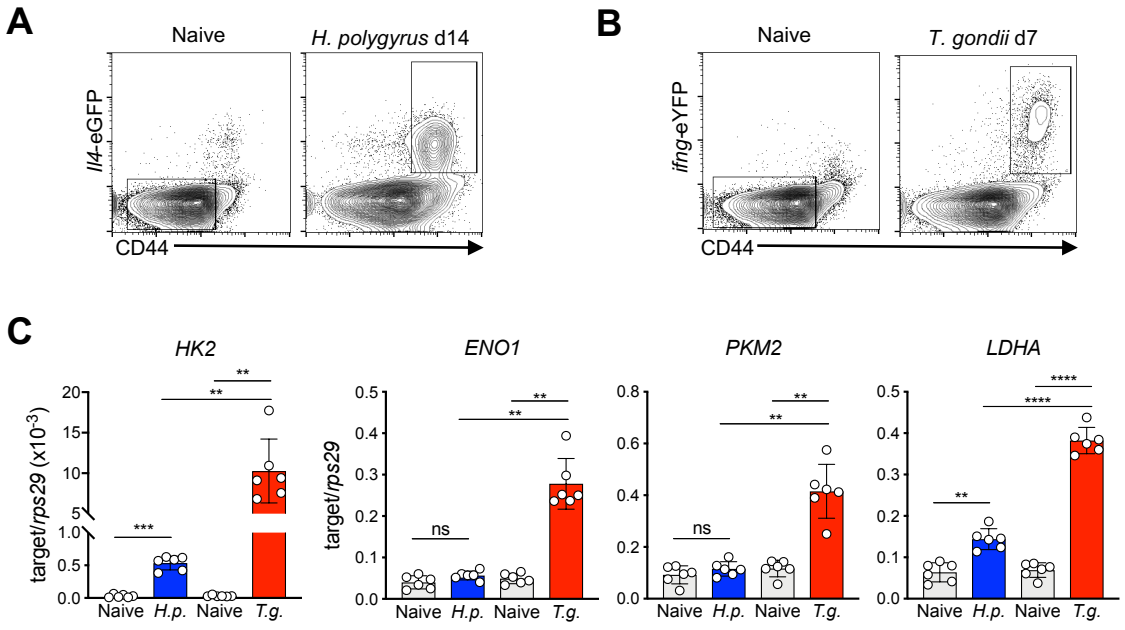

Figure 3.3 | Glycolytic gene expression is similar between anti-helminth Th2 and naïve CD4^{+} cells

A, B6.4get mice were infected with *H. polygyrus* and the resulting Th2 cells were isolated from the MLN at day 14 post-infection, alongside CD44^{Lo} naïve cells from MLNs of uninfected mice. **B**, GREAT mice were infected with *T. gondii* and at 7 days post-infection Th1 from infected MLNs and naïve cells from control MLN were cell sorted. **C**, Sorted Th1 and Th2 cells from infected mice were assessed for glycolytic gene expression by qPCR. Data shown are pooled from 2 independent experiments, n=3 mice per experiment.

To functionally correlate gene expression to metabolic output, *ex vivo* sorted Th1 and Th2 cells were subjected to Seahorse analysis (Fig. 3.4). Th2 cells from *H. polygyrus* infection had markedly low glycolysis compared to Th1 cells, irrespective of glucose or oligomycin treatment (Fig. 3.4A, B). The glycolytic rate of Th2 cells instead was more comparable to that of naïve $CD4^+$ cells following the administration of glucose. Th2 cells increased their ECAR in response to oligomycin, whereas naïve cells showed no increase (Fig. 3.4A, B). Th2 cells thus possess an increased glycolytic capacity relative to naïve cells, but the difference is slight compared to that of Th1 cells from *T. gondii* infection.

Following the provision of glucose, both Th1 and Th2 cells induced during infection displayed a comparable rate of mitochondrial metabolism, as measured by the OCR (Fig. 3.4C, D). Consequently, Th1 cells had a significantly depreciated OCR/ECAR ratio in comparison to naïve cells, reflecting the dramatic increase in Th1 glycolysis (Fig. 3.4E). In contrast, Th2 cells maintained a higher OCR/ECAR ratio that matched that of naïve $CD4^+$ cells (Fig. 3.4E). Furthermore, exposure to glucose promoted Th1 cells to reduce OXPHOS in favour of glycolysis but had no impact on Th2 OXPHOS, implying that oxidative metabolism in Th2 cells is regulated independently of glucose availability (Fig. 3.4C, D).

Altogether, Th2 cells isolated from an *H. polygyrus* infection fail to acquire a dominantly glycolytic phenotype. While they do possess an increased capacity to

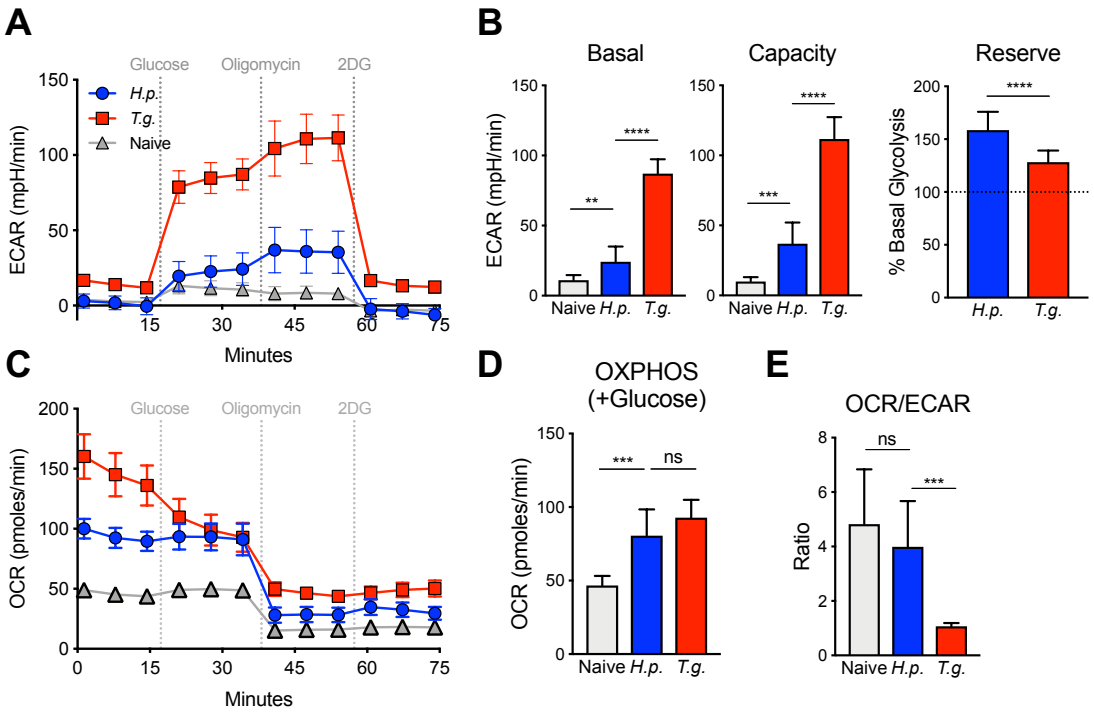

Figure 3.4 | Reduced glycolysis in Th2 cells during helminth infection

Sorted Th1 and Th2 cells from the MLN of *T. gondii* (*T.g.*) or *H. polygyrus* (*H.p.*) infected mice were assessed using the glycolytic-stress test. **A**, Real-time trace and **B**, graphical representation of the ECAR from *ex vivo* sorted cells. **C**, Simultaneous measurement of the OCR and **D**, graphical illustration of the OCR after glucose injection. **E**, Ratio of OCR over ECAR measured following exposure to glucose. Data pooled from 3 independent experiments. Graphed data calculated from highest measured values in each well.

engage glycolysis compared to naïve $CD4^+$ T cells, their use of glycolysis is much lower than that of *in vitro* activated cells, or *in vivo* activated Th1 cells.

3.2.3 Th2 metabolism is not strain dependent

Mice bred on different background strains have variable resistance to helminth infection[68]. With *H. polygyrus*, C57BL/6 background mice are susceptible and take several months to expel the adult parasites. BALB/c mice however expel worms more rapidly, within 1-2 months. This is in part due to a more robust Th2 response in the BALB/c mice, with a greater frequency of $CD4^+$ T cells producing a greater amount of IL-4[241]. Therefore, determining if there are metabolic differences from different strain backgrounds may give insight into the relationship between glycolysis and Th2 differentiation.

To address if the stronger Th2 response in BALB/c mice correlated with higher glycolysis, IL-4-expressing cells were isolated from the MLN of *H. polygyrus*-infected 4get mice on a BALB/c background. Metabolic analysis using the Seahorse glycolytic stress-test showed no distinguishable difference in the ECAR between Th2 cells isolated from either strain (Fig. 3.5A). These data suggest that differences in glycolysis do not appear to contribute to the enhanced Th2 response in BALB/c mice. There was also no appreciable difference in the OCR between strains (Fig. 3.5B). However, naïve cells from BALB/c background mice had a greater glycolytic rate relative to naïve C57BL/6 mice (Fig. 3.5A). Naïve cells may then have a smaller glycolytic threshold to reach in BALB/c mice, though the

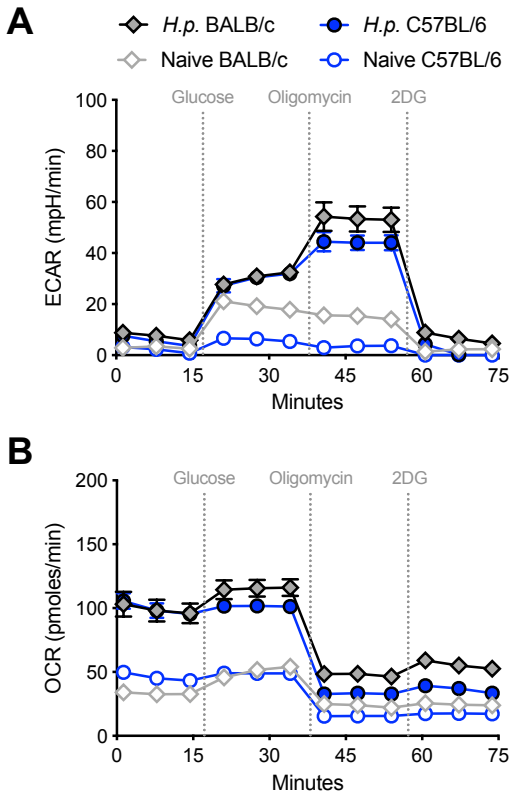

Figure 3.5 | Th2 glycolysis is not dependent on host background

4get mice on a BALB/c background were infected with *H. polygyrus* and MLN Th2 cells were purified 2 weeks later and subjected to the Seahorse glycolysis-stress test. **A**, ECAR and **B**, OCR of sorted cells compared to measurements from C57BL/6 4get mice, representative of 3 experiments (included in from Fig.3.4). Data from BALB/c 4get mice represents one experiment.

relationship between naïve cell metabolism and a predisposition to become activated has not been investigated. Despite the potential differences in naïve $CD4^+$ T cell metabolism, these data suggest that the low glycolytic rate in activated Th2 cells is not due to the strain background. Before being conclusive, the BALB/c data would need to be repeated as it represents only a single experiment.

3.2.4 Th2 glucose uptake is restricted *in vivo*

To eliminate the possibility that the metabolic differences measured between *in vitro*- and *in vivo*-generated Th2 cells were artefacts of technical perturbation (*i.e.* cell sorting following removal and processing of tissue), I next used a method in which I could quantify glucose uptake *in vivo* by injecting the fluorescent glucose analogue 2NBDG into infected mice. The emission spectrum of 2NBDG fluorescence overlaps with that of GFP/YFP, precluding the use of reporter mice for these experiments. Activated cells in the MLN were instead defined by CD44 expression (Fig. 3.6A). 2NBDG uptake in $CD4^+CD44^{Hi}$ cells from *H. polygyrus* infection was identical to that of $CD4^+CD44^{Lo}$ cells from naïve mice (Fig. 3.6B, C). In contrast, activated MLN cells from *T. gondii* infected mice had significantly increased glucose uptake (Fig. 3.6B, C). These experiments therefore validate the previous *ex vivo* data, suggesting that Th2 glycolysis is low *in vivo.*

Due to the potency of *T. gondii* infection in driving a type 1 response, I additionally assessed glucose uptake in $CD4^+$ cells activated during infection with influenza A virus (IAV). As IAV infects the airways, cells were isolated from the lung draining,

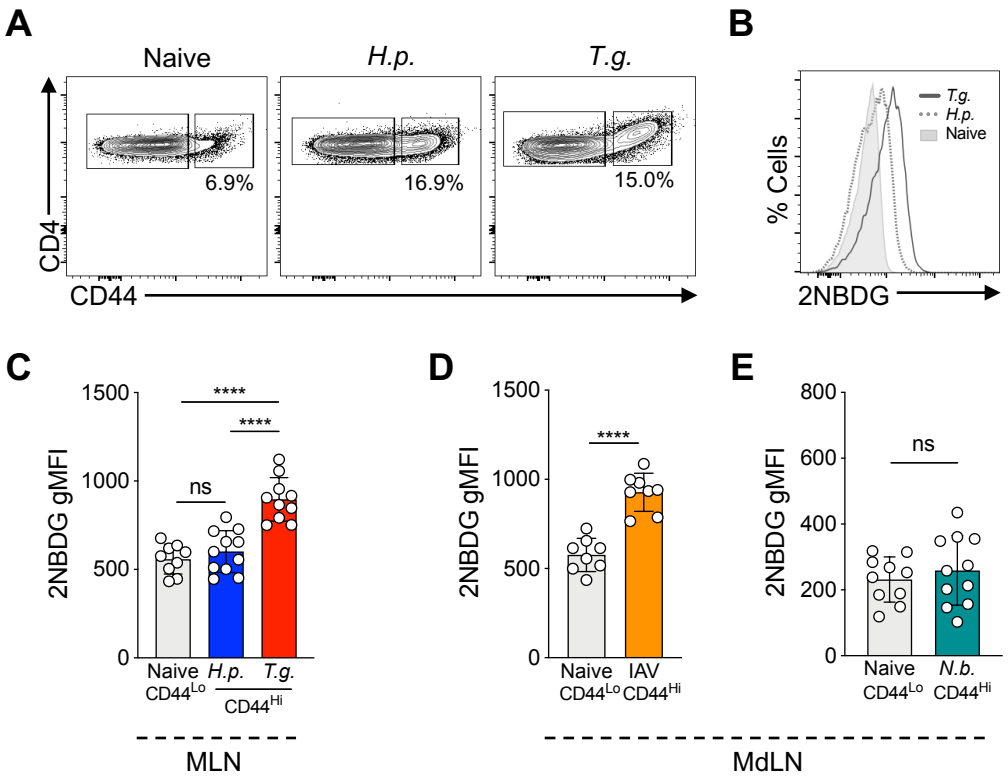

Figure 3.6 | CD4^{+} T cell glucose uptake increased *in vivo* from Th1 but not Th2 driving infection

Infected and naïve wild-type C57BL/6 mice were injected with 2NBDG one hour before sacrifice. **A**, Representative CD44 expression and gating for CD4^{+} cells from the MLN of naïve and *H. polygyrus* or *T. gondii* infected mice. **B**, Histogram showing 2NBDG fluorescence in CD44^{Hi} cells from the MLN of *H.p* and *T.g.* infected mice, and CD44^{Lo} cells from naïve controls. (**C**) Geometric mean fluorescent intensity (gMFI) of 2NBDG uptake in the MLN during *H.p.* and *T.g.* infection, and **D**, in the mediastinal LN during Influenza A virus (IAV) or **E**, *Nippostrongylus brasilensis* (*N.b.*) infection. Data points are individual mice, pooled from 3 independent experiments, n=2-4 mice per experiment.

mediastinal lymph node (MdLN). Therefore, this model was also used to assess if the initial infection site has an impact on $CD4^+$ T cell metabolism. A similar significant increase to that seen in *T. gondii* infection was evident between IAV and uninfected mice (Fig. 3.6D). 2NBDG uptake was further measured in the MdLN during *Nippostrongylus brasiliensis*, an additional model of helminth infection that migrates through the lung, generating a strong Th2 response. 2NBDG uptake in activated $CD4^+CD44^{Hi}$ cells in the MdLN was comparable to naïve $CD4^+CD44^{Lo}$ control cells, consistent with low Th2 glucose uptake (Fig. 3.6E).

Increased glucose consumption is therefore a shared quality of Th1 cells elicited from different sites of infection. Importantly however, glucose metabolism in Th2 cells during helminth infection is low *in vivo* as well as *ex vivo* and is not dependent on the initial site of infection.

3.2.5 Reduced proliferative capacity in Th2 cells

Glycolysis is intimately linked to cell-cycle entry and continued proliferation[141,146]. To test whether the low glucose metabolism observed in Th2 cells during infection was associated with a proliferative defect, harvested cells from infected reporter mice were stained for Ki67 expression. Indeed, despite having a similar frequency of cytokine positive cells in the MLN (Fig. 3.7A, B), Th2 cells from *H. polygyrus* infected mice had reduced Ki67 expression compared to Th1 cells from *T. gondii* infected mice (Fig. 3.7C, D). This indicates that, although differentiated and expressing cytokine, fewer Th2 cells are engaged in an active

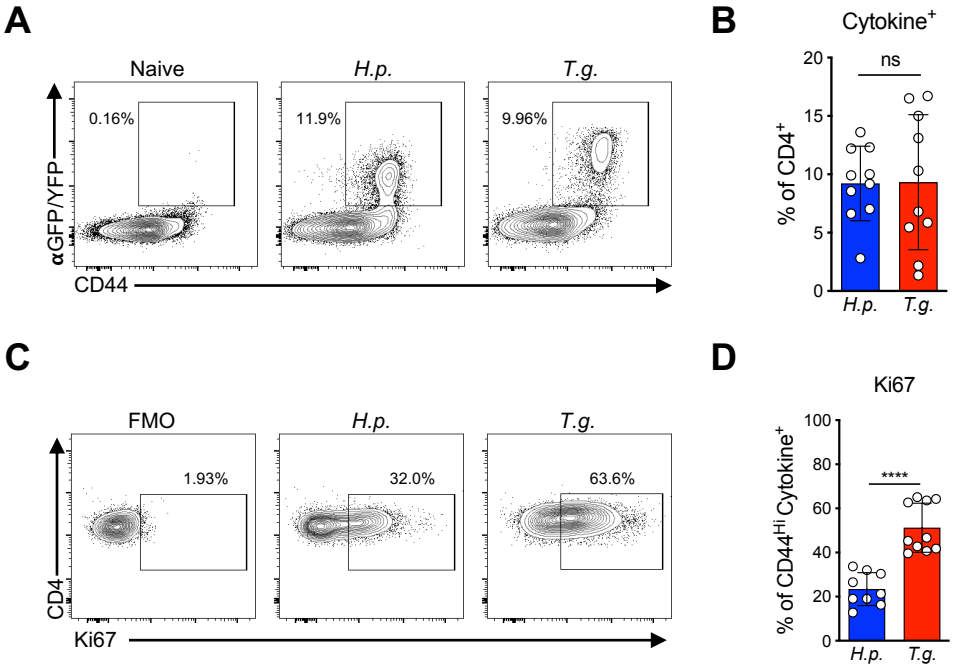

Figure 3.7 | Helminth-activated Th2 cells have reduced cell-cycle entry

After 1 week (*T. gondii*) or 2 weeks (*H. polygyrus*) post-infection, cells from the MLN were stained for Ki67 to assess cell-cycle entry. **A**, Representative staining of $CD4^+$ cells following fixation and GFP/YFP recovery. **B**, Frequency of reporter positive $CD4^+$ cells according to anti-GFP/YFP staining. **C**, Representative flow cytometry plots of nuclear Ki67 staining. **D**, Frequency of $Ki67^+$ cells within gated populations from *A*. Data pooled from 3 experiments with n=3-4 mice per group.

cell-cycle. Mainly, low Th2 glycolysis is consistent with an inability to fully support cell-cycle progression and cell division.

3.2.6 Glycolysis controls Th2 function

The observation that Th2 cells were modestly glycolytic was surprising given the current consensus that glycolysis is a critical requirement for Th2 cytokine production. To test the contribution of glycolysis to Th2 function, the HK2 inhibitor, 2-deoxy-d-glucose (2DG), was used to inhibit the pathway. Treating *in vitro*-polarized Th2 cells with a low dose of 2DG at the time of activation resulted in a reduction in *il4*-GFP, IL-5 and IL-13 expression (Fig. 3.8A, B). These data illustrate that blocking glycolysis is a potent method of abrogating Th2 function *in vitro.* Th1 cells were also tested for their ability to produce cytokine during 2DG treatment. As previously reported[146], Th1 cells were significantly inhibited in expression of IFNγ during glycolytic inhibition (Fig. 3.8C).

My data, shown previously, demonstrated the glycolytic activity of *in vivo* Th2 cells was very different to Th2 cells polarized *in vitro*. To investigate if glycolysis, though low, had an important role in driving the function of Th2 cells activated during infection, $CD44^{Hi}GFP^{+}$ cells from the MLN of *H. polygyrus* infected mice were sorted and re-stimulated with αCD3 in the presence or absence of 2DG for 3 days. Cytokine concentrations were then measured from the culture supernatants, which demonstrated reduced IL-4, IL-5 and IL-13 in 2DG treated cultures (Fig. 3.9A). Intracellular cytokine expression was also determined following PMA and

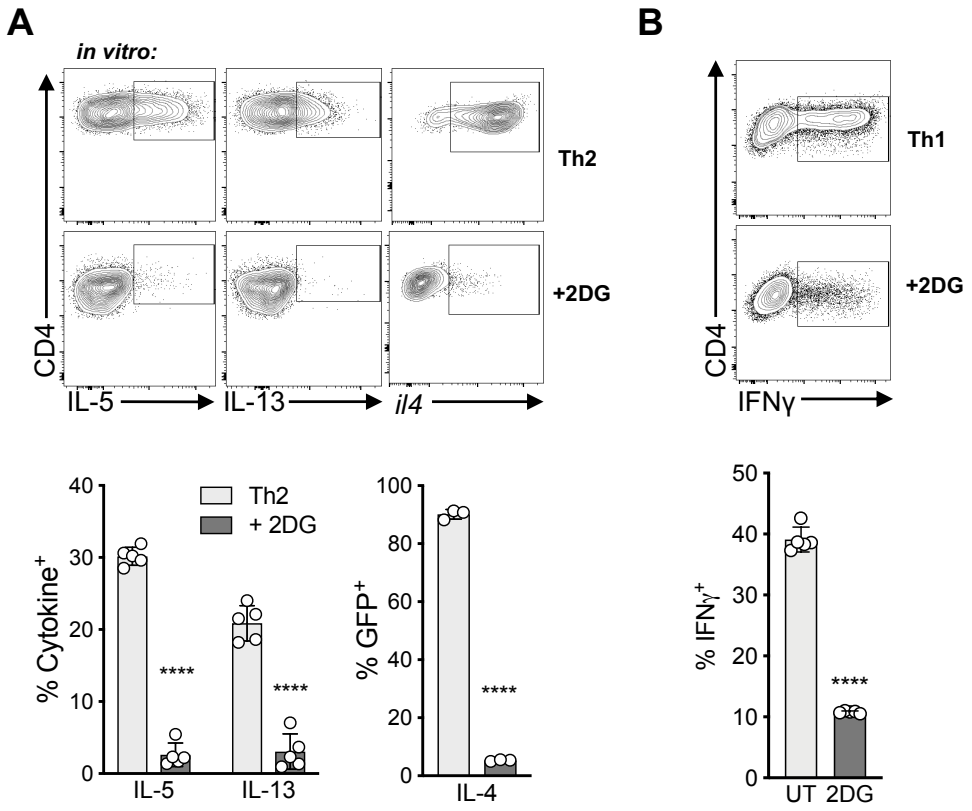

Figure 3.8 | Glycolysis is required for optimal Th2 cytokine production *in vitro*

In vitro CD4$^+$ T cells were polarized from B6.4get splenocytes in the presence or absence of 2DG to inhibit glycolysis at the time of primary activation. **A**, Representative staining and frequencies of intracellular cytokines and GFP expression in Th2 polarized cells. **B**, Intracellular staining and frequency of IFNγ$^+$ Th1 cells. Representative of at least 3 independent experiments.

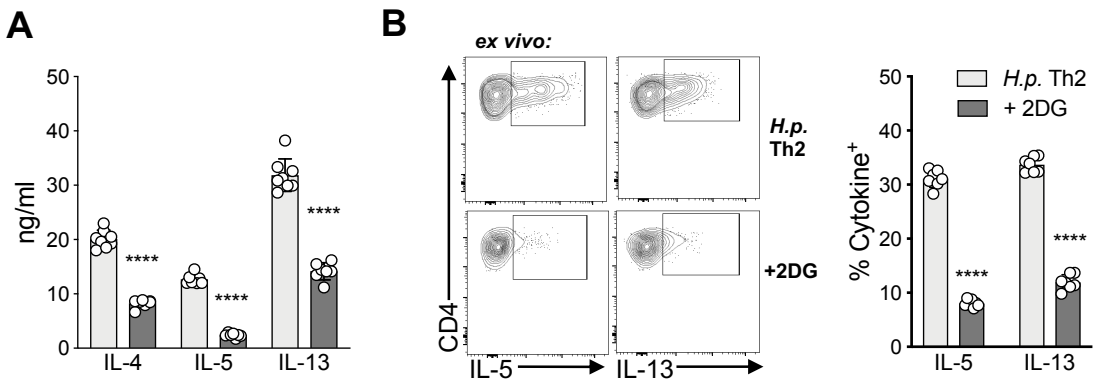

Figure 3.9 | Inhibiting glycolysis during *ex vivo* re-stimulation blocks Th2 cytokine expression

GFP$^+$ IL-4-expressing cells were sorted from the MLN of *H. polygyrus* infected B6.4get mice and stimulated with αCD3 for 3 days with or without 2DG. **A**, Supernatant cytokine concentrations, determined by cytometric bead array after 3 days of *ex vivo* culture. **B**, Representative intracellular cytokine staining and frequency of cytokine-expressing cells after 5 hours of PMA/ionomycin stimulation with monensin. Data shown representative of at least 3 independent experiments.

ionomycin stimulation, which similarly indicated defects in cellular IL-5 and IL-13 expression after 2DG treatment (Fig. 3.9A, B). Together, these data emphasize that glycolysis is an important metabolic pathway, *in vitro* and *in vivo,* for promoting Th2 cytokine production and secretion. Interestingly, however, Th2 cells maintain an almost naïve-like glucose metabolism during helminth infection.

3.3 Discussion

Previously, *in vitro* studies have fostered a consensus that Th2 cells are the most glycolytic $CD4^+$ T cell subset[121,175,202]. At present, no studies have confirmed this using *in vivo* models. Manipulation of metabolism using transgenic mouse models suggests that glycolysis is indeed important for *in vivo* Th2 differentiation, for example by deletion of the mTOR adaptor protein raptor[192]*.* CD4-specific knockout of the *rptor* gene blocked metabolic reprogramming, abrogated Th2 cytokine production, and prevented cell infiltration into the lung during a house dust-mite model of allergic asthma. Therefore, using different infection models to generate Th1 and Th2 responses in mice, I hypothesized Th2 cells would be highly glycolytic, as is seen using *in vitro* models[176,192].

When selectively analysing Th1 or Th2 populations from infected mice based on *ifng* or *il4* expression, the contrary was observed. During *H. polygyrus* infection, activated Th2 cells displayed similar gene expression patterns to naïve $CD4^+$ T cells for glycolytic enzymes, while Th1 cells from *T. gondii* infection significantly

up-regulated these genes. Real-time measurements of metabolism demonstrated that Th2 do maintain a glycolytic rate higher than that of naïve cells, but to a much lower degree than Th1 cells. Direct *in vivo* injection of a fluorescent glucose analogue showed that, in contrast to Th1 cells, Th2 cells fail to increase glucose uptake during activation. This implies Th2 cells maintain a low glycolytic rate compared to Th1 cells, at least in part, due to reduced glucose entry into the cell. This is in stark contrast to my observations made using *in vitro* cultures, which fit the literary consensus that Th2 are a highly glycolytic subset.

The low glycolytic rate in Th2 cells during *H. polygyrus* infection calls to question whether increased glycolysis during activation is necessary for Th2 differentiation or function. This was tested by activating naïve cells *in vitro* or re-stimulating *in vivo*-derived Th2 cells with low concentrations of 2DG to inhibit hexokinase, the first enzyme in glycolysis. In both cases 2DG significantly reduced the frequency of cells capable of producing cytokines. These data indicate that although glycolysis remains low during *H. polygyrus* infection, hexokinase activity is still required for continued cytokine expression *ex vivo.* Therefore, some increase in glycolytic activity still seems to be needed to facilitate optimal Th2 function.

Despite being widely used, several reports have highlighted off-target effects of 2DG[243,244], thus the data discussed above must be interpreted cautiously. The 2DG target, HK2, is also a rheostat for mTORC1 activation and downstream autophagy[245], both of which have non-redundant effects on T cell behaviour[246,247].

Alternative activation of macrophages is inhibited by 2DG via a glycolysis-independent mechanism that impairs OXPHOS[243]. However, lower concentrations of 2DG, below 1 mM, reduce glycolysis without reducing OXPHOS in macrophages. Similar concentrations have also been used for T cells, without impeding activation or proliferation[192]. I therefore used a concentration of 0.5 mM to reduce the likelihood of off-target effects. Cytokine expression in Th2 cells was nearly abolished at this low concentration indicating high sensitivity to hexokinase inhibition.

In spite of the observations that glycolysis is necessary for maximum cytokine production, Th2 cells possess a low glycolytic metabolism *in vivo*. If blocking glycolysis inhibits Th2 function, what is the reason for Th2 cells maintaining low glycolysis during helminth infection? A potential explanation could be that, *in vivo*, glucose is less readily accessible to Th2 cells. Interestingly, helminth infection, or direct administration of the canonical Th2 cytokine IL-4, precipitates hypoglycaemia in mice, suggesting activated T cells in the MLN may be faced with a glucose-depleted environment[248,249]. A lack of available glucose would be a logical justification for low glycolytic activity in Th2 cells, and also agrees with my observation that Th2 cells had reduced glucose-uptake during infection.

An alternative possibility that could explain low glycolysis in Th2 cells *in vivo* is differences in cognate TCR signal strength in response to pathogen-derived antigens. Given that the TCR provides the major T cell activating signal, it is

unsurprisingly also a major driver of metabolic adaptations in T cells[150,236]. Glucose uptake in $CD4^+$ T cells increases dose-dependently with titrated concentrations of activating αCD3[141]. Several early reports on Th2 dynamics suggested that low dose antigen or weak affinity for peptide-MHC complexes promotes Th2 polarization *in vitro*[250–252], and the same has been suggested more recently *in vivo*[99,101,253]. If a lower threshold of TCR stimulation is required to drive Th2 differentiation, then Th2 cells may be activated with a TCR strength that elicits only small changes in glycolysis. As the antigens that drive immunity to *H. polygyrus* are as of yet unidentified, this hypothesis is challenging to test in the context of infection. Van Panhuys *et al*[253] demonstrated that TCR avidity could bias $CD4^+$ T cell polarization using mice with transgenic TCRs that bind the same antigen with differing strengths. Comparing the metabolic features of these different TCR populations after antigen recognition could be used to test the hypothesis that weaker TCR signals are associated with reduced metabolic activity, and potentially with Th2 polarization, *in vivo*.

Although glycolysis remains low in Th2 cells *in vivo*, data from myself and others indicate a necessity for glycolysis for Th2 cytokine production. To date, few studies have aimed to uncover mechanisms relating cellular glycolysis to Th2 function. It has been proposed that glucose dictates Th2 differentiation by regulating surface expression of IL-4Rα[192]. Naïve $CD4^+$ T cells activated in low glucose media failed to upregulate surface expression of IL-4Rα, and expression increased dose-dependently with added glucose. These concepts fit partially with my data

indicating that Th2 cells acquire less glucose and have reduced proliferative capacity in *H. polygyrus* infection, as well as the notion that helminth infected mice may be hypoglycaemic[248,249]. However, the argument that glucose availability controls Th2 polarization can be countered by previous observations demonstrating that IL-4Rα becomes highly expressed on naïve bystander cells in the MLN during *H. polygyrus* infection[241]. As these cells do not experience TCR stimulation, it is unlikely they would undergo metabolic changes associated with activation, and therefore would not increase glucose uptake. Furthermore, a glucose concentration of 0.05 mM was sufficient to induce IL-4Rα, well below physiological murine blood glucose levels of 3 to 6mM, which would suggest sufficient glucose is available for maximal receptor expression[248,249]. Additionally, my data show that glucose metabolism is low in $CD4^+$ cells already expressing IL-4, indicating polarization towards Th2 cells has occurred. This does not exclude the possibility that glucose concentration in the draining lymph node negatively regulates Th2 cytokine production, but it is unlikely to be via IL-4Rα dependent regulation.

Few studies have addressed a mechanistic relationship between metabolism and Th2 cells. In contrast, several studies have elucidated connections between metabolism and Th1 function, which can be used to provide some speculation about Th2 cells. In Th1 cells, it has been shown that the glycolytic enzyme GAPDH can tether IFNγ mRNA during low glycolytic activity by binding the 3'-untranslated region (UTR), preventing translation[124,146]. When the glycolytic rate was high,

GAPDH instead bound its glycolytic substrate, and mRNA was released. Thus, a high glycolytic rate was required to permit IFNγ translation. Accordingly, my data show that treatment with 2DG obstructs IFNγ production in Th1 cells. Whether GAPDH also plays a role in Th2 cytokine regulation was never tested, but post-transcriptional regulation has been demonstrated for Th2 cytokines. It has been shown that both IL-4 and IL-13 mRNA can be sequestered by 3'-UTR binding proteins[254,255], so a similar relationship between GAPDH and Th2 cytokine is therefore plausible.

Metabolism has also been recently connected to epigenetic modifications in immune cells[256]. In Th1 cells, glycolysis has an additional role in promoting IFNγ expression by providing a free acetate pool to be utilized for epigenetic histone modification[195]. Supplementing Th1 cultures with acetate after restricting glycolysis via LDHA deficiency was sufficient to restore IFNγ expression. Acetate treated cells lacking LDHA had an increased enrichment for permissive histone marks at the *ifng* locus that were absent in untreated cells. Whether glycolysis in Th2 cells has an epigenetic consequence has not been tested. In the context of my work, Th2 cells were analysed based on transcriptional reporting for *il4,* hence already epigenetically primed for IL-4 cytokine production. As a high glycolytic rate is already absent in these cells, it suggests an epigenetic modification is a less likely link between glycolysis and Th2 function. However, IL-5 and IL-13 expression is differentially regulated from that of IL-4[43], and may require further investigation for epigenetic and post-transcriptional control.

Interestingly, while Th2 cells from infected mice showed a minimal increase in glycolysis, relative to Th1 cells, OXPHOS was similar in both subsets. In Th2 cells, however, OXPHOS was unaffected by glycolysis, as the OCR was maintained when glucose was added to the system. Th1 cells, on the other hand, had a sharp decrease in the OCR after glucose addition, suggesting a preference for glycolysis over OXPHOS. The stability of the OCR in Th2 cells may indicate a greater dependence on mitochondrial metabolism over glycolysis. In support of this hypothesis, one study assessing the role of mitochondrial respiration in $CD4^+$ T cells found deletion of the mitochondrial protein Tfam rendered cells unable to maximize oxygen consumption, leading to a compensatory increase in glycolysis[257]. In this setting, T cells polarized in Th2 conditions were impaired in IL-4 expression and instead adopted Th1 characteristics, such as T-bet expression and IFNγ secretion.

It could then be hypothesized that an over-active glycolytic pathway is detrimental to Th2 function. Work interrogating the role of HIF1α in T cells, a positive regulator of several glycolytic genes, supports this notion. Constitutive stabilization of HIF1α leads to increased glycolysis in T cells[182]. During house-dust mite challenge, a canonical Th2 model, active HIF1α instead elicited a potent and uncharacteristic Th1 response in the lung[182]. Conversely, deletion of HIF1α has no apparent impediment to Th2 differentiation, at least *in vitro*[177]. Hence, while my data suggest glycolysis is a requisite for Th2 cytokine expression, unchecked glycolysis appears more favourable to Th1 differentiation, possibly at the expense of Th2. In line with

a concept that high glycolysis hinders Th2 differentiation, the glycolytic rate of Th2 cells from helminth infection remains more similar to that of naïve $CD4^+$ T cells than activated Th1 cells from *T. gondii* infection (Fig. 3.4).

A limitation of present discussions within immuno-metabolism is that they often refer to glycolytic manipulation as if it is an all-or-none effect, but it is more likely that the need for glycolysis lays on a continuous scale, with each subset relying on an optimal range for differentiation and continued cytokine expression. Depending on the scenario, altering glycolysis may need a minor or major shift to generate a functional effect on the T cell response. My data suggest that only a minimal increase in T cell glycolysis is necessary to progress Th2 differentiation. Beyond the early stages of activation however, Th2 cells may once again switch their reliance from glycolysis to OXPHOS. Accordingly, the late addition of 2DG to Th2 cultures has been shown to have negligible effects on cytokine expression[192]. However, in the context of pathogenicity, the failure to down-regulate glycolysis may be a factor in promoting inappropriate Th2 activation. T cells stimulated *ex vivo* from people or mice with asthma, or malnourished mice, become preferentially Th2 cells and are associated with a higher than normal glycolytic rate, compared to healthy or naïve controls [191,206,258]. Therefore, the increased glycolytic ability of these cells may partially account for the unwanted activation of Th2 cells in certain diseases.

Overall, I have shown that while highly glycolytic *in vitro,* Th2 cells maintain a low glycolytic rate *in vivo* during helminth infection. Th1 cells, however, retain their dominantly glycolytic phenotype *in vitro* and *in vivo* during protozoan infection. Inhibiting glycolysis both *in vitro* and *ex vivo* results in a decrease in Th2 cytokine production, which identifies glycolysis as a necessary facet of Th2 function. Despite this observation, data from others have hinted that OXPHOS may shoulder a more dominant role in fuelling Th2 activity, and that unrestrained glycolysis is obstructive to Th2 differentiation.

Chapter 4: Helminth imposed regulation of Th2 metabolism

4.1 Introduction and rationale

An effective anti-helminth response relies on the activation and polarization of $CD4^+$ T helper 2 (Th2) cells[259]. The production of cytokines IL-4, IL-5 and IL-13 by Th2 cells promotes worm killing through the activation of macrophages, eosinophils and mast cells, and contributes to worm expulsion by inducing goblet cell hyperplasia, mucus secretion and epithelial shedding within the intestine[259]. However, helminths actively alter the T cell response to their advantage[67]. Chronic helminth infection is often associated with an increased frequency of suppressive regulatory T cells, and antigen-specific Th2 cells have been shown to adopt a "hypo-responsive" state, both contributing to long-lived parasite survival in the host[71,260,261]. While this down-regulation of the T cell response is detrimental to the host's ability to eliminate infection, current research is actively applying the same regulatory properties of helminths to treating patients suffering chronic immune disorders[65]. However, our understanding regarding the beneficial mechanisms of helminth-based therapies is far from complete.

Whether or not helminth manipulation of the T cell response involves metabolic regulation is not yet known. Metabolism is already an appreciated target of manipulation by viruses and bacteria. Induction of host cell glycolysis is a prevailing requirement for viral replication upon entry into the cell[262]. A recent study

on HIV-1 interestingly discovered that viral replication induces exosome formation in host cells which then condition bystander T cells to increase glycolysis and promote viral spread[263]. Similarly, intracellular pathogenic bacteria can tailor host cell metabolism to promote a favourable environment. Immunity to *Mycobacterium tuberculosis* requires the successful activation and phagocytosis by macrophages. These processes are dependent on macrophages engaging glycolysis, but the bacterium is capable of surviving within the phagosome by inducing metabolic quiescence[264]. Accordingly, inhibiting glycolysis in macrophages leads to unrestrained bacterial growth[265]. These examples illustrate that manipulating host metabolism is a common method used by intracellular pathogens to promote successful infection. Whether large extracellular agents, such as helminths, can apply similar mechanisms to modulate immunity is an interesting question.

I have previously shown that Th2 cells activated *in vivo* during *H. polygyrus* infection maintain a low rate of glycolysis (Chapter 3). Nonetheless, my data also indicate that glycolysis is still required for optimal Th2 cytokine production. Infection with *H. polygyrus* potentiates a regulatory response that effectively suppresses the parallel Th2 response. This is achieved in part by Treg induction[71], as both cytokines and co-inhibitory molecules expressed by Tregs have been associated with negative regulation of glycolysis, such as IL-10 production and the expression of co-inhibitory receptors[218,266]. Additionally, Rick Maizels and colleagues have characterized the major protein families excreted/secreted by *H. polygyrus,* and many of the constituent proteins have putative metabolic

functions[267]. I therefore predicted that *H. polygyrus* is able to actively regulate T cell metabolism to dampen glycolysis and Th2 function, indirectly by expanding Tregs, or directly through the production of suppressive molecules.

4.2 Results

4.2.1 Th2 metabolism is independent of Treg regulation

I first aimed to determine the role of Tregs in controlling Th2 metabolism. To prevent Treg expansion during *H. polygyrus* infection, I administered the αCD25-blocking antibody PC61 to mice one day before infection. The frequency of $Foxp3^+$ cells was successfully reduced in the MLN of infected mice on day 14 of infection (Fig. 4.1A). Treg depleted mice had an increased frequency of activated $CD44^{Hi}$ cells (Fig. 4.1B), as well as a similar increase in IL-4-expressing cells in the MLN (Fig. 4.1C), compared to isotype-injected controls. Therefore, as anticipated, Tregs contributed to restraining the anti-helminth T cell response. Th2 cells sorted from mice receiving either PC61 or the isotype control, however, had equivalent ECAR traces when tested by the Seahorse glycolysis stress-test (Fig. 4.1D). Hence in *H. polygyrus* infection regulatory T cells impede the Th2 response, but not through control of glycolysis.

4.2.2 Helminth products restrain Th2 function but not differentiation

I next sought to determine if helminths possess the ability to directly alter Th2 function by producing suppressive molecules. $CD4^+$ splenocytes from B6.4get

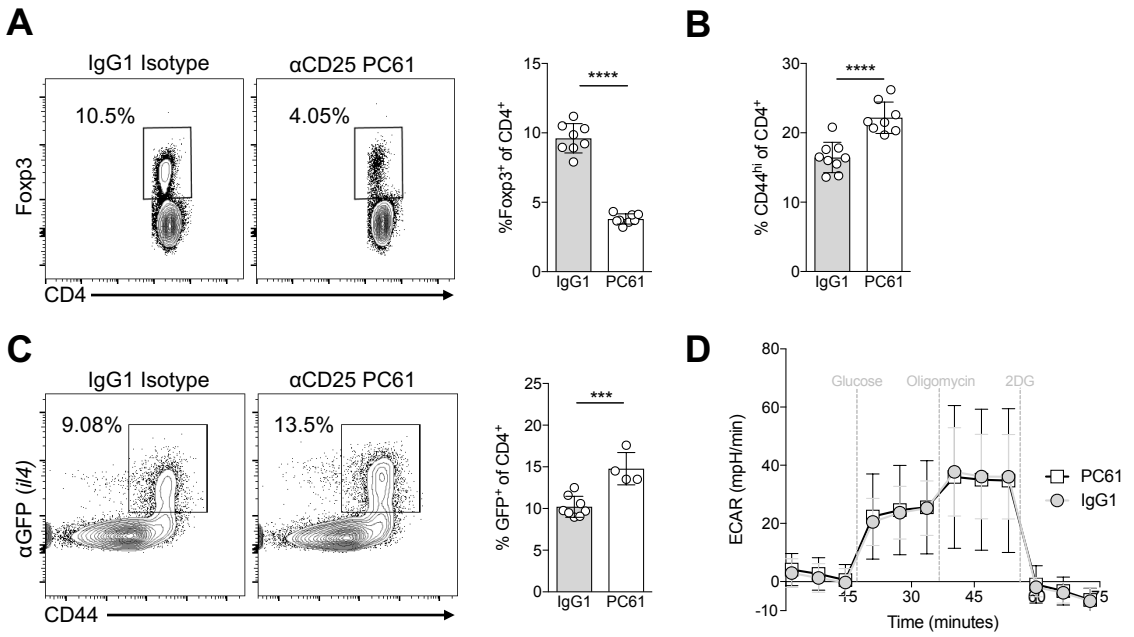

Figure 4.1 | Th2 glycolysis is not controlled by regulatory T cells

B6.4get mice were injected with 1 µg PC61 antibody or IgG1 isotype one day before *H. polygyrus* infection. Day 14 post-infection frequency of **A**, Foxp3^{+} cells, **B**, GFP-*il4* expressing cells and **C**, CD44^{Hi} cells were determined, data representative of 3 independent experiments. **D**, *il4*-eGFP^{+} cells were sorted and analyzed using the Seahorse glycolysis-stress test, 3 combined experiments are shown.

mice were stimulated via αCD3 and αCD28 in Th2 polarizing conditions in the presence of total unfractionated excretions/secretions from *H. polygyrus* (HES). Compared to untreated Th2 cultures, HES significantly inhibited *il4*-eGFP, as well as intracellular IL-5 and IL-13 expression (Fig. 4.2A). Cytokine expression was reduced independently of T cell activation as Th2 cells treated with HES displayed a similar blasting profile (Fig. 4.2B) and CD44 expression (Fig. 4.2C). Helminth products therefore have the ability to functionally impair Th2 cells.

HES has been shown to include molecules that drive Treg induction, which have been shown to be metabolically different from other Teff types. To check whether Th2 cultures treated with HES contained more Tregs, which could indicate they have different overall metabolic qualities compared to untreated Th2 cultures, we measured the expression of the Th2 transcription factor GATA3 and the Treg transcription factor Foxp3. Cultures treated with HES had comparable GATA3 expression to untreated controls, indicating both treatment groups had similar potential to differentiate towards Th2 cells (Fig. 4.3A). Analysis of Foxp3 expression showed that Treg expansion roughly doubled in cultures polarized in the presence of HES, but the frequencies of $Foxp3^+$ cells remained relatively low, increasing from a mean of 2.6% to 6.2% (Fig. 4.3B).

Ultimately, metabolic analysis will be performed on sorted *il4*-expressing cells. I selectively analysed transcription factor expression within the *il4*-$eGFP^+$ population from B6.4get cultured $CD4^+$ splenocytes. GATA3 expression was

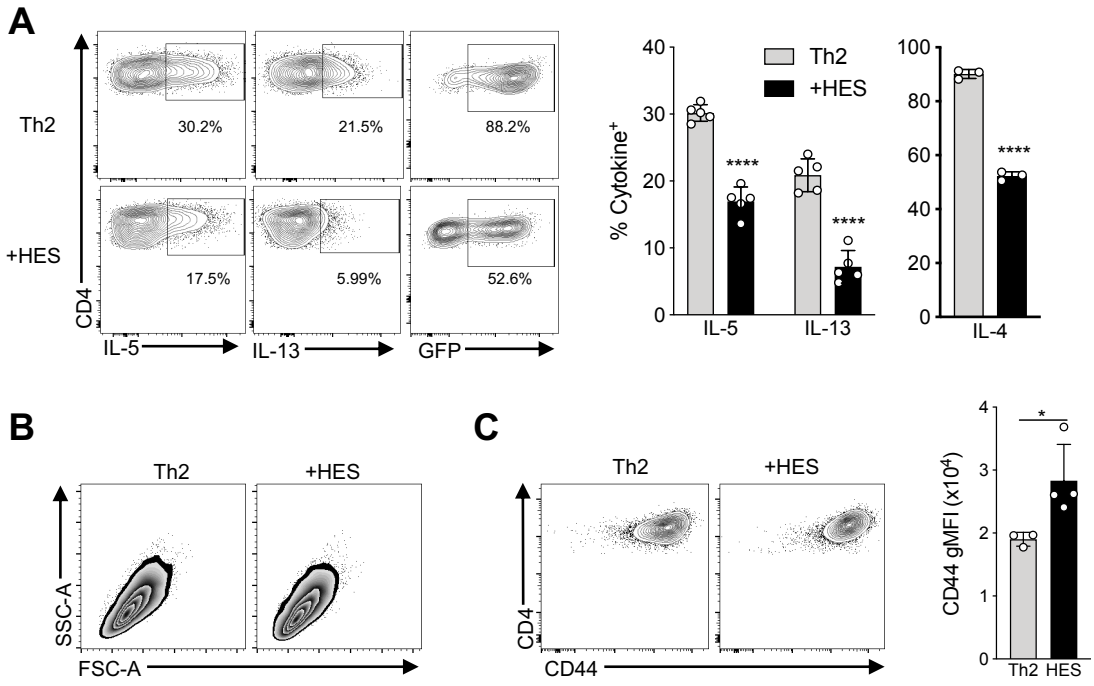

Figure 4.2 | Helminth-derived products alter CD4$^+$ cytokine production

CD4$^+$ isolated splenocytes were activated *in vitro* and treated with or without 5 µg/ml of total unfractionated HES. **A**, Representative intracellular IL-5 and IL-13 staining, and live GFP-reporter expression for IL-4, and frequency of cytokine expressing cells in Th2 polarized cultures. **B**, Blasting profile and **C**, comparison of CD44 for HES treated and untreated CD4$^+$ T cells 4 days post-activation. Representative of 3 experiments.

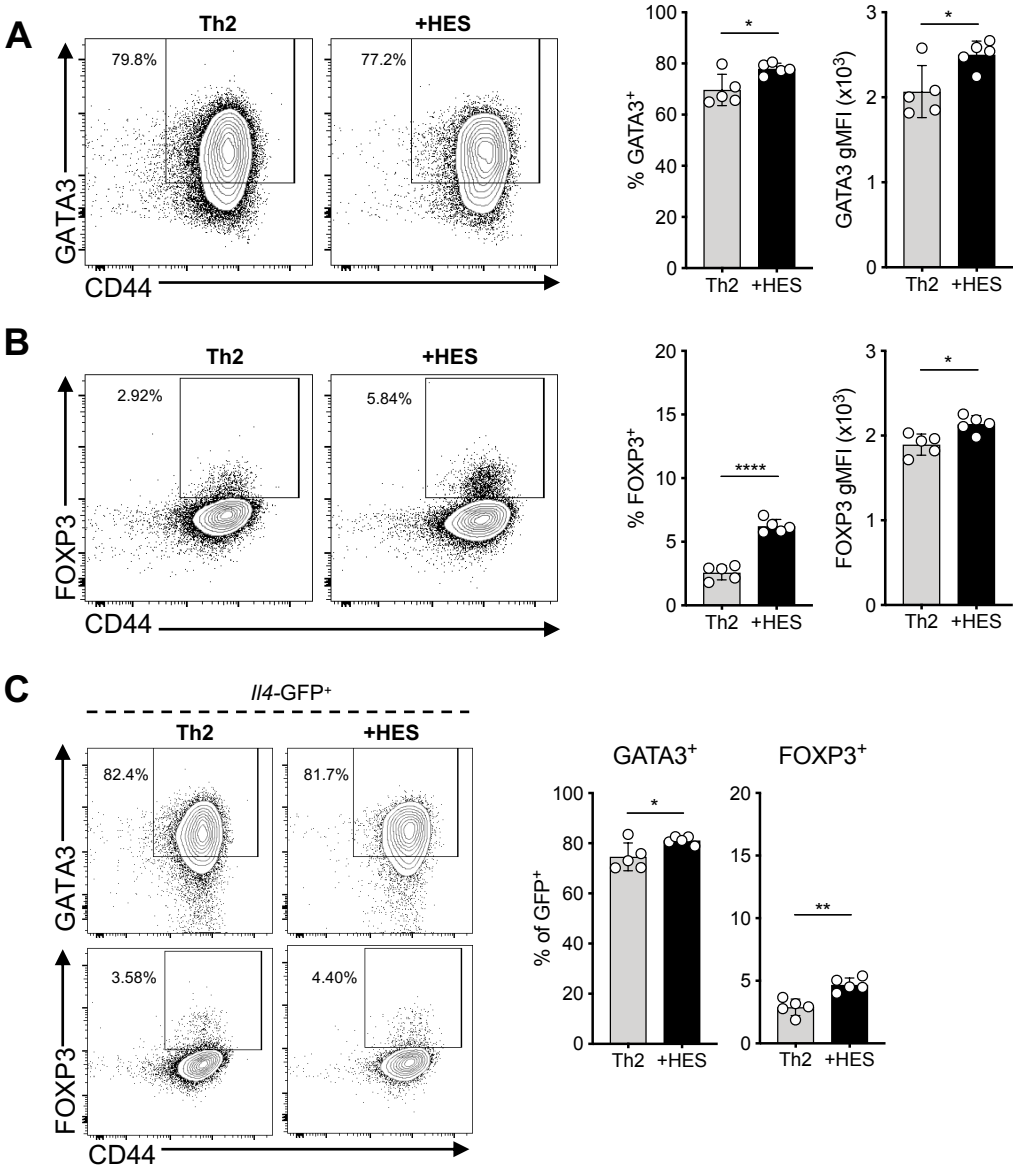

Figure 4.3 | Stable transcription factor expression in Th2 cells treated with HES

Total CD4$^+$ cells were assessed for (**A**) GATA3 and (**B**) Foxp3 expression after 4 days of culture in Th2 conditions with the presence or absence of HES. (**C**) Analysis of GATA3 and FOXP3 expression after selecting for GFP (*il4*) expressing Th2 cells. Representative of 2 experiments.

similarly comparable in HES-treated and untreated *il4*-expressing populations (Fig. 4.3C). The frequency of $Foxp3^+$ cells was still increased within the GFP^+ cells exposed to HES (Fig. 4.3C). However, the difference between control and HES treated groups was reduced slightly, with 2.9% and 4.7% $Foxp3^+$ cells present in control and treated cultures, respectively.

Altogether these data imply HES mediates only minor changes in transcription factor expression under Th2 polarizing conditions. Thus, potential changes in metabolism are unlikely to be a consequence of altered differentiation pressure imposed by molecules in HES. Furthermore, as HES reduced *il4*-eGFP, IL-5 and IL-13 expression, but not GATA3 expression, these observations indicate HES may block cytokine production separately from differentiation.

4.2.3 HES inhibits mTOR signalling *in vitro*

To gauge if the reduction in cytokine expression imposed by HES was associated with a reduction in T cell glycolysis, I examined the strength of mTOR activation, a central regulator of T cell metabolism[247]. mTOR signalling occurs through two complexes, mTORC2 and mTORC1, leading to phosphorylation of the targets Akt and S6, respectively, as outlined in Figure 1.6. Th2 cultures were treated with HES at the time of primary activation. 4 days post-simulation, cells were either re-stimulated with PMA or left unstimulated before fixation, and phosphorylated mTOR targets were assessed by flow cytometry.

Without secondary PMA stimulation, no differences were observed in p-Akt between Th2 cultures with or without HES (Fig. 4.4A). However, compared to HES-treated cells, PMA stimulation yielded a significant increase in the frequency, and strength, of phosphorylated Akt in Th2 cells activated in the absence of HES (Fig. 4.4A). Before secondary stimulation, S6-phosphorylation was increased in untreated Th2 cells, however the frequency of p-S6 positive cells was maximized in both conditions subsequent to PMA stimulation (Fig. 4.4B). In spite of cells ubiquitously exhibiting S6 phosphorylation after restimulation, the degree of phosphorylation in each cell was significantly reduced in cells exposed to HES (Fig. 4.4B). Therefore, *H. polygyrus* secretes molecules with the capacity to reduce mTOR signalling, suggesting T cell glycolysis may be a target of immune suppression.

4.2.4 Reduced mTOR signalling in helminth-activated T cells

To relate these findings to the *in vivo* T cell response, I determined the degree of mTOR signalling in activated $CD4^+$ T cells from *H. polygyrus* and *T. gondii* infection. Prior to this analysis, I observed that activated T cells from the MLN of *H. polygyrus* infected mice were physically smaller than those activated from *T. gondii* infection (Fig. 4.5A). Cell size has been previously connected to the extent of mTOR activation[268]. Therefore, these data suggested that activated T cells induced by *H. polygyrus* may have impaired mTOR signalling relative to *T. gondii*-activated T cells.

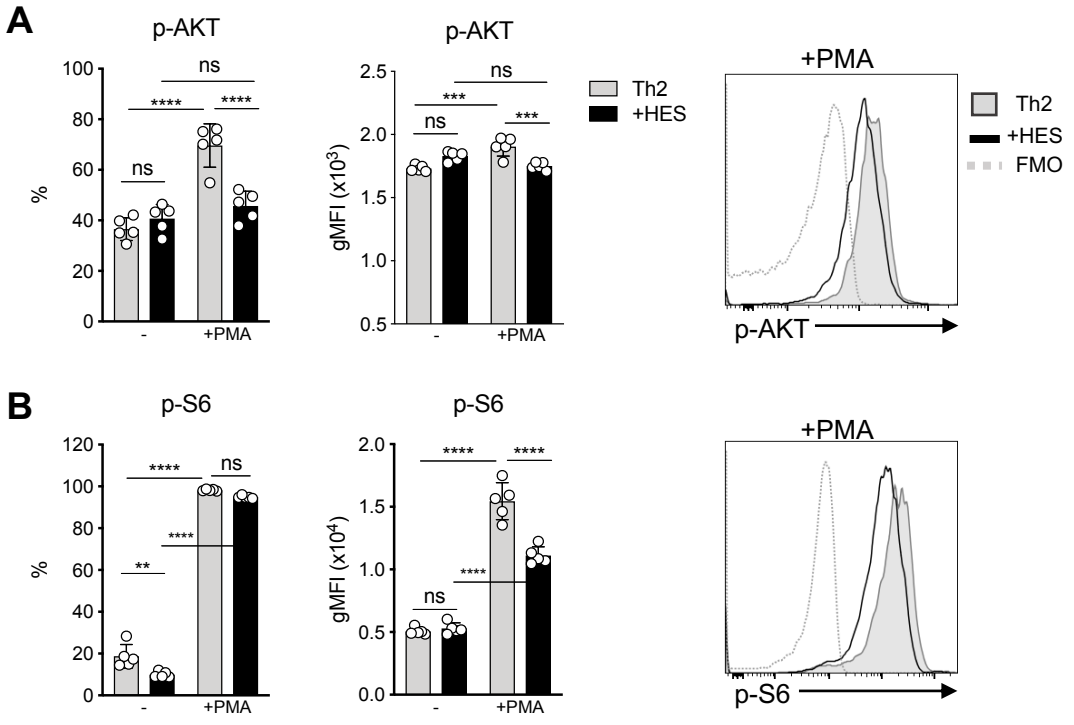

Figure 4.4 | mTOR is signalling is regulated by HES in vitro

Th2 polarized cultures with or without HES were stained and analyzed 4 days post-stimulation for phosphorylated targets of mTOR. Cells were either fixed immediately or stimulated with 50 ng/ml of PMA for 30 min. Frequency, gMFI and representative histograms of **A**, p-AKT and **B**, p-S6 within live single $CD4^+$ cells. Representative data of 4 independent experiments.

Differences in mTOR signalling between infections were determined by comparing the fold change of fluorescent intensity in $CD44^{Hi}$ relative to $CD44^{Lo}$ cells from the same MLN. As predicted, $CD44^{Hi}$ cells from *T. gondii* infected mice had a greater increase in Akt phosphorylation, compared to the increase seen in *H. polygyrus* infection or mice left uninfected (Fig. 4.5B). Although $CD44^{Hi}$ cells during *H. polygyrus* infection displayed an increase in p-Akt compared to $CD44^{Lo}$ cells, the increase was similar to that observed for $CD44^{Hi}$ in unchallenged animals.

The activated $CD4^{+}$ T cell population from *T. gondii* similarly exhibited a significantly greater increase in p-S6 compared to those from helminth infected or naïve mice (Fig. 4.5C). During *H. polygyrus* infection, $CD44^{Hi}$ cells had minimal changes in S6 phosphorylation compared to $CD44^{Lo}$ cells from the same MLN (Fig. 4.5C). Conversely, $CD44^{Hi}$ cells from the MLN of naïve animals still demonstrated an increase in p-S6, which was significantly greater than the fold change seen in *H. polygyrus* infection (Fig. 4.5C).

Activated T cells in helminth infection therefore exhibit reduced mTOR signalling, determined in regard to both mTORC1 and mTORC2, with respect to *T. gondii* infection. In particular, mTORC1 signalling was lower in *H. polygyrus*-activated T cells than in the $CD44^{Hi}$ population from experimentally unchallenged mice. This observation could denote active suppression by the helminth parasite, and further argues that T cell metabolism is a potential target of *H. polygyrus in vivo.*

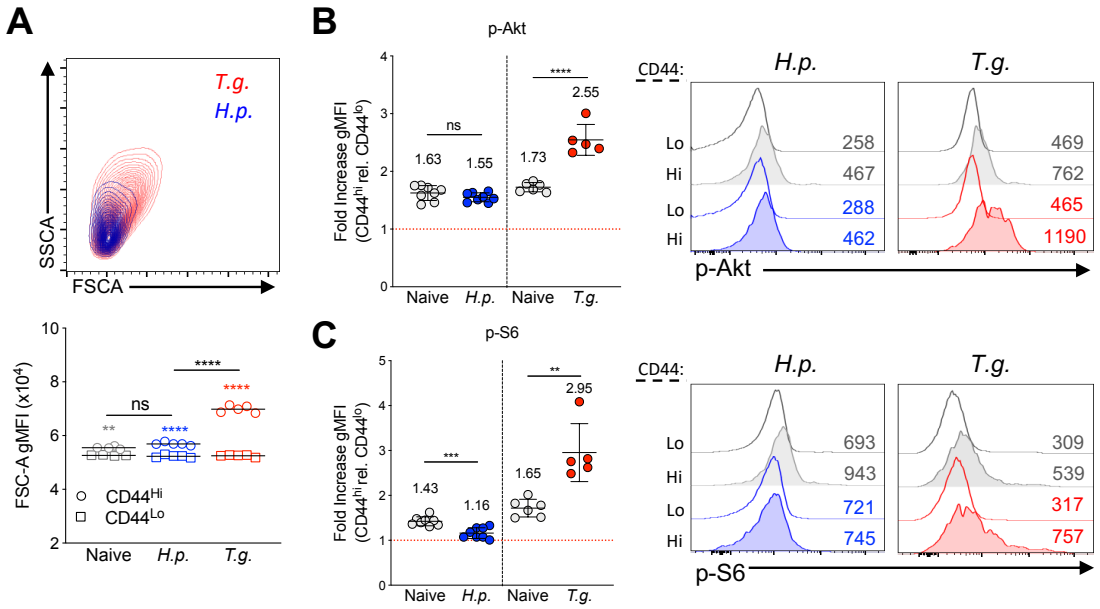

Figure 4.5 | Restricted mTOR signalling in helminth-activated CD4⁺ T cells

C57BL/6 mice were infected with *H. polygyrus* for 14 days or *T. gondii* for 7 days before harvesting the MLNs. **A**, Blasting profile of $CD4^{+}CD44^{Hi}$ cells (top) and mean forward-scatter area of $CD44^{Lo}$ and $CD44^{Hi}$ cells. **B** and **C**, MLNs were immediately processed into fixation buffer and stained for phosphorylation targets of **B**, mTORC2 (p-Akt) and **C**, mTORC1 (p-S6). Data are presented as the relative gMFI of $CD44^{Hi}$ cells compared to $CD44^{Lo}$ cells within the same sample. *A* is representative of 3 independent experiments, *B* and *C* are pooled data from 2 experiments, n=2-4 mice per group, per experiment.

4.2.5 Direct impact of HES on Th2 glycolysis

I next asked whether HES can directly alter glycolysis in Th2 cells. $CD4^+$ cells from B6.4get splenocytes were activated and polarized for 4 days before cell-sorting *il4*-GFP^+ cells from untreated and HES-exposed, Th2 cultures. Sorted Th2 cells were assessed for metabolic activity via the Seahorse glycolysis-stress test. In an initial experiment, the inclusion of HES resulted in Th2 cells with reduced basal and maximal glycolytic rates, shown by the ECAR, compared to untreated Th2 cells. This result suggested that helminth products may indeed possess the ability to inhibit Th2 glycolysis (Fig. 4.6A).

However, for reasons I have been unable to identify, subsequent difficulties with my *in vitro* polarizations meant that cultures no longer yielded sufficient numbers of viable Th2 cells to repeat this analysis. Successful restoration of Th2 polarizations was achieved, after multiple attempts of re-optimization, by using a different clone of αCD3 for stimulation. Using the new clone, I repeated the Seahorse glycolysis-stress test on Th2 cells treated with or without HES. After testing sorted Th2 cells from restored cultures for differences in glycolysis, none were distinguished between sorted HES-treated or untreated groups (Fig. 4.6B).

Therefore, it appears *H. polygyrus* derived molecules may have the ability to manipulate Th2 metabolism, though effects are inconsistent between experiments. One observation made when activating Th2 cells with the alternate αCD3 clone was that cells activated and proliferated more rapidly, evident from

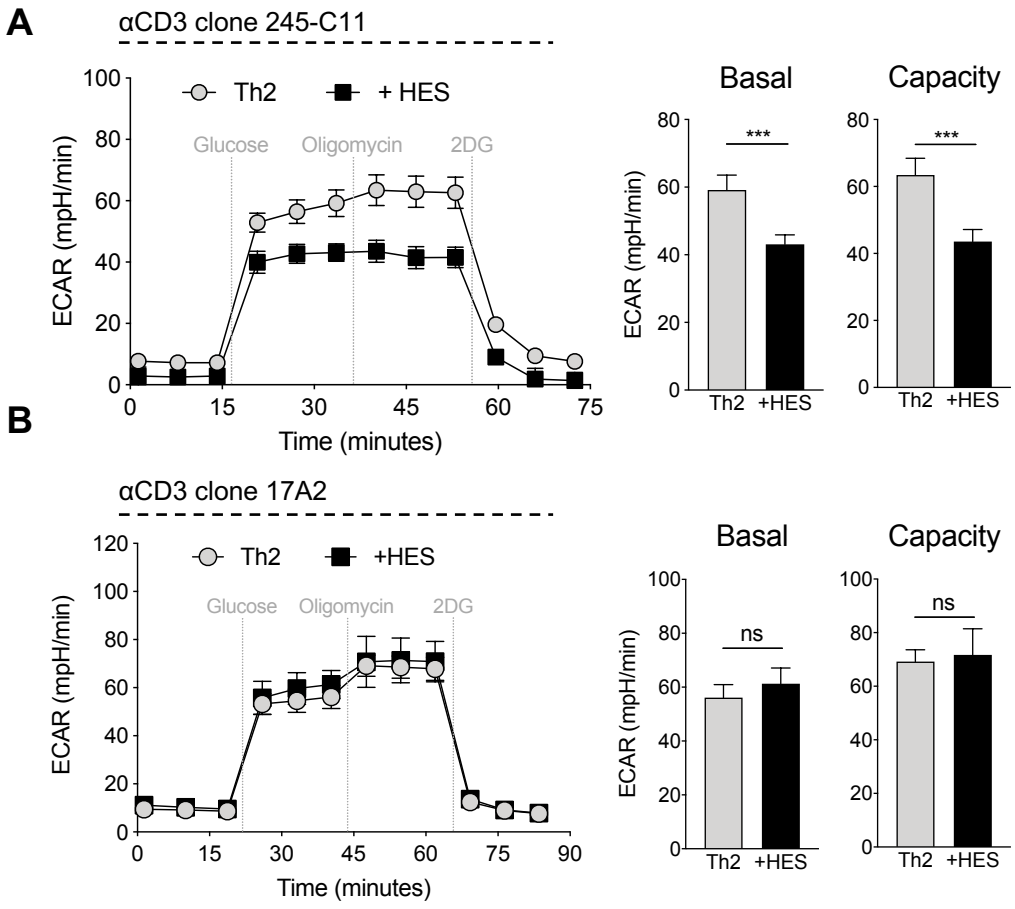

Figure 4.6 | Variable impact of HES treatment on Th2 glycolysis *in vitro*

Th2 cells were polarized *in vitro*, in the presence or absence of 5 µg/ml of HES, sorted after 4 days of culture according to *il4*-eGFP expression and analyzed via the Seahorse glycolysis stress-test. Naïve $CD4^+$ T cells were stimulated with 1µg/ml of **A**, hamster anti-mouse CD3ε clone 245-C11 or **B**, rat anti-mouse CD3 complex clone 17A2.

increased yellowing of media and visible proliferation clusters. The variation between experiments might suggest that the effect of HES, at the concentration used, is bordering a suppressive threshold to inhibit glycolysis. The stronger activation observed when stimulating naïve $CD4^+$ T cells with the newer clone might indicate it provides a more potent TCR stimulus. Stronger activation could correlate with a more dramatic change in glycolysis, and therefore diminish the outcome of HES treatment. More optimisation could be done with varying TCR signal strength, or concentrations of HES, in order to further elucidate any potential suppressive effects of helminth products on T cell glycolysis.

4.2.6 A helminth-derived TGF-b mimic suppresses Th2 glycolysis

The proteomic landscape of HES includes several hits with potential metabolic implications[267]. Of particular interest is the recently characterized TGF-β mimic, *Hp*-TGM. Recent work on the TGF-β cytokine has revealed it can down-regulate cellular glycolysis in a variety of immune cells[269–271]. *Hp*-TGM shares many of the same signalling pathways as TGF-β[272], so I therefore investigated if *Hp*-TGM can alter glycolysis in $CD4^+$ T cells under Th2 polarizing conditions.

Hp-TGM or TGF-β were added to *in vitro*-activated cultures and *il4*-expressing cells were sorted 4 days later and analysed using the Seahorse. Both TGF-β and TGM severely inhibited *il4* expression in Th2 cultures (Fig. 4.7A, B). Despite this, the few *il4*-$eGFP^+$ cells remaining were still specifically sorted for analysis. Th2 cells sorted from TGM or TGF-β cultures showed an overlapping reduction in

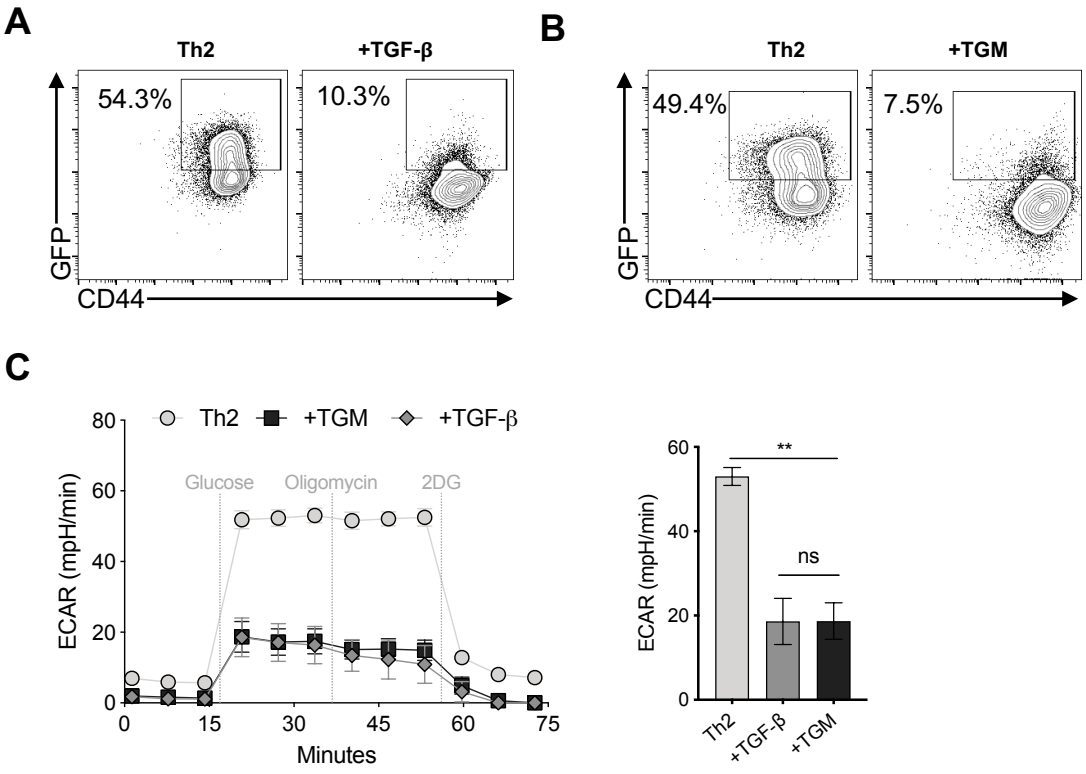

Figure 4.7 | *Hp*-TGM phenocopies TGF-β inhibition of glycolysis

In vitro Th2 cultures from B6. 4get splenocytes cultured with (**A**) 20 ng/ml TGF-β or (**B**) 20 ng/ml *Hp*-TGM. (**C**) *il4*-expressing $CD4^+$ cells from Th2 cultures with or without TGF-β or TGM were sorted and analyzed using the glycolysis stress-test. Data shown are indicative of 2 separate experiments.

glycolysis compared to untreated Th2 cells (Fig. 4.7C). Therefore, *H. polygyrus* secretes a molecule, *Hp*-TGM, that can alter $CD4^+$ T cell metabolism when tested *in vitro*, which may be a contributing factor to the potential changes in glycolysis imposed by total HES.

4.3 Discussion

Host metabolism is a common target amongst different pathogenic species that rely on the intracellular microenvironment of a host cell[262,264,273]. Whether large multicellular helminth parasites also target host metabolism has not been investigated. Given that immunity to *H. polygyrus* is critically dependent on IL-4 production from the T cell response[259], which is limited by glycolytic-restriction (Fig. 3.8 and Fig. 3.9), in this chapter I tested the ability of *H. polygyrus* to dampen Th2 function and asked whether the outcome was connected to glycolytic inhibition.

H. polygyrus infection causes the expansion of peripherally-derived Treg numbers in the MLN that are functionally suppressive of the Th2 response[71]. Tregs are a reservoir for IL-10 and TGF-β production[274], immuno-suppressive cytokines that have been shown to block metabolic signalling that promotes increased glycolysis[266,269]. Co-inhibitory molecules on the surface of Tregs, such as CTLA-4, additionally block glycolytic metabolism during T cell activation[125,218]. My first hypothesis was that the suppressive mechanisms enacted by Tregs could regulate

Th2 metabolism during *H. polygyrus* infection. Even though Treg depletion allowed a greater expansion of activated *il4*-expressing $CD4^+$ T cells in the MLN, the glycolytic phenotype remained identical between Treg sufficient and deficient mice, suggesting that the low glycolytic rate observed in Th2 cells *in vivo* is not a result of Treg mediated suppression.

The failure to observe a reduction in Th2 glycolysis in the absence of Tregs, however, could be due to insufficient Treg depletion. I used an antibody mediated method to ablate Tregs prior to infection. While this was successful at reducing Treg frequencies, a notable population of Tregs was still present at 2 weeks post-infection (Fig. 4.1A). A more complete ablation of Tregs, such as that achieved by using a Foxp3 driven diphtheria toxin receptor, could potentially reveal a role for Tregs in suppressing metabolism. However, removing Tregs this way leads to "immune chaos" within the $CD4^+$ T cell response to *H. polygyrus*, associated with unchecked expansion of IFNγ-secreting cells, while Th2 cytokine production was unaffected[71]. Hence, if T cell metabolism is regulated by Tregs during infection, it may be a more important factor in dampening potentially harmful Th1 responses. This is a logical proposition given that *H. polygyrus* causes barrier breach in the intestinal wall as larvae burrow into the submucosa and back out into the lumen[68], potentially giving commensal microbes a window to trigger an inflammatory response. Nevertheless, my data indicate that low glycolysis is a feature of Th2 cells in *H. polygyrus* infection independent of suppressive signals originating from Tregs.

My next hypothesis included the potential that *H. polygyrus* can produce factors directly capable of altering $CD4^+$ T cell glycolysis, and thus the Th2 response. I first tested this by adding total HES to *in vitro* cultures, determining its impact on Th2 polarization and cytokine production. $CD4^+$ T cells had comparable activation in the presence of HES, but IL-4, IL-5 and IL-13 production was diminished compared to untreated T cells. Surprisingly, GATA3 expression was also comparable in the presence or absence of HES, suggesting that Th2 function but not differentiation was altered by HES. $Foxp3^+$ Tregs can be directly induced by HES in non-polarizing conditions[272], however in Th2 cultures Foxp3 was generally similar between treatment groups, with a minor increase following exposure to HES (Fig. 4.3). The consistency in transcription factor expression suggested that reduced cytokine production in Th2 cells could be due to other mechanisms separate from their ability to differentiate. I therefore anticipated impaired Th2 cytokine production would be due in part to restricted glycolysis.

Glycolysis is regulated by mTOR signalling[247]. My analysis of mTOR signalling further supported that glycolysis could be a regulatory target of *H. polygyrus*. Th2 cells activated *in vitro* had impaired phosphorylation of the mTORC1 and mTORC2 targets, S6 and Akt respectively, when cultured in the presence of HES. Differences were most pronounced for p-S6. Interestingly, mTORC1 has been found to be strongly associated with promoting anabolic metabolism and glycolysis, whereas the role of mTORC2 in metabolic regulation is less well

defined[247]. Hence reduced mTORC1 signalling would be expected to correlate with a reduced glycolytic rate.

In vivo, $CD4^+$ T cells activated in response to *H. polygyrus* had significantly weaker S6 and Akt phosphorylation than T cells responding to *T. gondii*. It therefore appears that Th2 glycolysis during *H. polygyrus* infection is reduced, in part, due to reduced mTOR signalling. Furthermore, S6 phosphorylation was reduced in *H. polygyrus* infection compared to naïve mice. Though the particular factors driving a $CD44^{Hi}$ population in the unchallenged animals used for these experiments are not known, infected mice were housed similarly, thus the same factors should have been present. Despite this, $CD44^{Hi}$ cells from the MLN of *H. polygyrus* infected mice displayed almost no difference in p-S6 compared to $CD44^{Lo}$ cells, whereas cells from naïve mice had increased p-S6. In conjunction with my *in vitro* data showing HES inhibits p-S6, this could be interpreted to mean mTOR signalling, particularly through mTORC1, is actively suppressed during *H. polygyrus* infection.

Several pieces of evidence point towards an inhibitory effect of *H. polygyrus* on host cell glycolysis. Earlier characterization of the major protein families excreted/secreted by *H. polygyrus* suggested many of the produced proteins have functions related to metabolism[267]. Hexokinase, aldolase and enolase, all enzymes in the glycolysis pathway, are found in the top 100 expressed proteins in HES, suggesting glycolysis may be a key target of suppression. However, when

directly evaluating the glycolytic rate of Th2 cells polarized *in vitro*, HES treatment had variable outcomes. One experiment showed a significant reduction in Th2 glycolysis during HES treatment, though this has not yet been successfully repeated. This may be a consequence of switching αCD3 clones. TCR stimulation strength is one of the most determinant signals controlling T cell metabolism *in vitro*[141,142,150]. Therefore, if the two clones have distinct activation potentials, the difference could have a masking effect on the suppressive effects mediated by HES. In light of other indirect evidence gathered, there is still a sufficient foundation to maintain the hypothesis that HES is suppressive of Th2 glycolysis. Support for this hypothesis may be revealed by optimizing the degree of TCR stimulation. A more thorough approach would be to fractionate HES and test the ability of concentrated fractions to alter T cell glycolysis. Successful glycolytic inhibition by certain fractions could then be used to identify particular factors that are responsible, as has been done previously for identifying helminth products with immunological activity[272].

Following the potential, albeit inconsistent, changes in Th2 glycolysis directed by *H. polygyrus* products, I tested the possible contribution of *Hp*-TGM to alter metabolism. TGF-β has been shown to alter glycolysis in NK cells[271], macrophages[270] and T cells[275], via an mTOR dependent mechanism. During primary T cell activation, smad-2/3 phosphorylation downstream of TGF-β signalling interferes with CD28 induced signals[269]. *Hp*-TGM has been shown to ligate the same receptors as TGF-β and activates smad-2/3 with similar

effectiveness[272]. By testing their effects on *in vitro* Th2 cultures, I found both molecules were strongly suppressive of *il4* expression, and though transcription factors were not assessed, the high concentrations used likely promoted polarization towards either Th9 or Treg phenotypes[272]. Consistent with published studies, TGF-β significantly reduced glycolysis in sorted *il4*-eGFP$^+$ cells. Moreover, sorted cells from *Hp*-TGM treated Th2 cultures had a reduced glycolytic rate identical to those treated with TGF-β. Therefore, *Hp*-TGM has robust inhibitory action on CD4$^+$ T cell glycolysis as well as Th2 function.

Ultimately, *in vitro* systems represent extremes that are rarely observed in a physiological capacity. My data demonstrate this may particularly be true in the context of Th2 cells, which have low glycolysis *in vivo* but are highly glycolytic when polarised artificially in culture. Therefore, when testing the ability of helminth molecules to suppress Th2 glycolysis *in vitro*, the strength of activation, and associated unnaturally high glycolytic rate, could require much greater suppressive activity to see an effect on glycolysis. For example, in experiments testing the effect of HES, 5 μg/ml was used, a concentration previously shown to induce Treg polarisation during unbiased CD4$^+$ T cell activation. When testing *Hp*-TGM, 20 ng/ml was used, which is estimated to be a minimum of 20-times the concentration found in HES[272]. With this estimation, 5 μg/ml of HES would possess less than 1 ng/ml of TGM, a concentration that has little impact on Th2-polarized cultures[272]. The collection of HES additionally relies on the incubation of free-living parasites in culture media, and its contents might not then be truly representative

of what the helminths secrete when inhabiting a host. Despite these considerations, *H. polygyrus* is able to drive Treg induction in adoptively transferred TCR transgenic $CD4^+$ cells *in vivo*, and HES is able to mitigate *in vivo* inflammatory T cell responses to allogenic transplant, and to allergic challenge[74,272,276]. Admittedly, HES is a milieu of proteins and metabolites that could have multiple functional implications, but these examples illustrate that particular molecules (*i.e. Hp*-TGM) can still have a biologically significant impact *in vivo*, even if low concentrations are ineffectual *in vitro.* This may also be true of additional molecules that target T cell glycolysis. In order to test this further, a similar transgenic TCR adoptive transfer model could be used, in which recipient mice are either infected with *H. polygyrus* or injected with HES near the time of challenge with cognate transgenic TCR antigen[74]. Adoptively transferred transgenic T cells could then be isolated and metabolically analysed.

Altogether, the results presented in this chapter suggest that molecules produced by *H. polygyrus* may affect host Th2 metabolism. The data at this point are not definitive, however, and require further investigation to support the notion that HES is able to suppress $CD4^+$ T cell glycolysis during Th2 polarization. If this hypothesis is further supported after more experimentation, the resulting data could then be evidence for a novel set of interactions between parasites and host. These interactions are predicted to be favourable for helminth longevity within the host, and therefore could potentially represent future targets to be exploited therapeutically.

Chapter 5: A tissue-based metabolic checkpoint for Th2 function

5.1 Introduction and rationale

Th2 cytokine production is locally regulated at the tissue site during immune challenge[42,43]. IL-4 secretion is furthermore temporally segregated from IL-5 and IL-13 expression[43]. While production of IL-4 is initiated in the draining lymph node, IL-5 and IL-13 production is restricted to barrier sites after receiving further instruction from innate cells and environmental cues from the affected tissue. This spatial regulation creates non-overlapping roles for the Th2 cytokines IL-4 and IL-13 despite their common signalling through the IL-4R[277], as helminth infection is cleared normally in IL-4$^{-/-}$ mice, but not IL-13$^{-/-}$ mice[21,54]. In the periphery, Th2 cell cytokine expression can be promoted by ILC2s that present peptide-MHCII[82] and produce an early source of canonical Th2 cytokines to amplify the ensuing CD4^{+} T cell response once cells reach their target[80]. In conjunction, damaged endothelial and epithelial cells release alarmin cytokines in the form of TSLP, IL-25 and IL-33 which signal directly to both ILC2s and CD4^{+} T cells resulting in local cytokine production by Th2 cells[278].

Amongst the alarmins, IL-33 is of particular importance to Th2 development[78]. Though all three alarmins cooperate to promote an optimal Th2 response, a single knock-out of the IL-33 receptor (ST2, or IL1RL1) during helminth infection caused the strongest reduction in eosinophilia compared to single deficiencies of IL-25 or

the TSLP receptor[43]. A variety of T cell types are able to transiently express the IL-33 receptor, but constitutive expression is only observed in Th2 cells and GATA3^{+} Tregs[279]. Accordingly, the Th2 transcription factor GATA3 is required for ST2 expression[280]. Functionally, IL-33 signalling is sufficient to induce TCR independent cytokine production in primary and memory Th2 cells[42,281]. In helminth infection IL-33 promotes T cell dependent clearance of worms, while IL-33 deletion is associated with a defective Th2 response and increased parasite fitness[73,282–284]. The importance of IL-33 is further highlighted by the recent discovery that *H. polygyrus* produces its own biological inhibitor of IL-33, of which the purified form is capable of abrogating the lung Th2 response to allergens[72]. As IL-4, IL-5 and IL-13 expression has been shown to rely on glycolysis (Fig. 3.8), the strong induction of Th2 cytokines by IL-33 might indicate it could have role in promoting appropriate metabolic adaptations in Th2 cells entering the tissue.

Although glycolysis has been suggested to have important roles in Th2 cell activation and function, a shift towards fatty acid (FA) consumption is the most common metabolic trait observed in innate cells during type 2 challenge. Metabolic phenotyping of ILC2s from the homeostatic intestine revealed they rapidly acquire extracellular fatty acids, more so than other ILC subsets[285]. Similarly, AAM increase FA uptake in an IL-4/STAT6 dependent manner[166,286]. In AAM these fatty acids fuel mitochondrial metabolism and are required for macrophage function and helminth expulsion[132]. The metabolic transition of AAM is in part dependent on expression of the transcription factor PPARγ, a positive regulator of FA uptake and

FA oxidation (FAO)[166]. Although FA metabolism has yet to be assessed in Th2 cells *in vivo*, multiple studies have elucidated that expression of PPARγ in effector $CD4^+$ T cells is specific to the Th2 subset[231,287]. T cell specific knockout of PPARγ inhibits Th2 cell polarization and resistance to helminth infection. These data hint that FAO may also be an attribute of Th2 cell metabolism.

My initial characterization of Th2 cell metabolism during *H. polygyrus* infection assessed cells from the draining lymph node during T cell priming and showed that glycolysis of these cells remains low (Chapter 3, Fig. 3.3-6). Evidence for tissue-dependent regulation of Th2 function[42,43] suggests that metabolism may be regulated between lymph node egress and arrival at the site of infection, allowing an up-regulation of glycolysis to permit cytokine production. Furthermore, the prevalence of high FAO in alternatively activated innate cells prompted me to ask if FAO is also a property of adaptive Th2 cells. In this chapter I determined if Th2 cells undergo a metabolic checkpoint following migration to the tissue, assessing both glucose and FA metabolism. I further hypothesized that IL-33 signalling may be a critical driver of any metabolic changes.

5.2 Results

5.2.1 Glycolytic gene expression remains low in lung Th2 cells

To examine Th2 cells from the draining lymph node as well as from the effector tissue, I used an alternative model of helminth infection, *Nippostrongylus brasiliensis*. The prolific mucus secretion induced by *H. polygyrus* in the infected

intestine makes it technically difficult to isolate viable cells from the tissue, hence I needed a different system to assess $CD4^+$ T cells at priming and effector sites. After sub-cutaneous infection with *N. brasiliensis* larvae, the parasite migrates through the lung, generating a type 2 response, before being transmitting to the gut via the airways (Fig. 1.2). Therefore, using B6.4get mice to examine IL-4-expressing cells, the Th2 response could be evaluated in the draining mediastinal lymph node (MdLN) and in the lung (Fig. 5.1).

Metabolic differences between $CD4^+$ T cells from the MdLN or the lung were initially assessed using qPCR to analyse metabolic gene expression. MdLN and lung Th2 cells were sort-purified according to CD44 and *il4*-eGFP expression from infected mice, using the gating strategy shown in Fig. 5.1. Simultaneously, uninfected animals were used to obtain $CD44^{Lo}$*il4*-$eGFP^-$ naïve T cells from both tissues as a comparison (Fig. 5.1).

Previously, I found that Th2 cells primed in the MLN during helminth infection used minimal glycolysis, but that glycolysis was needed for optimal cytokine production. One explanation for these discordant observations could be that glycolysis is regulated in the periphery where cytokine production is strongest. I hypothesized that tissue-based immune signals would promote glycolysis to enhance localized cytokine production by lung-migrated Th2 cells. As previously shown for *H. polygyrus* (Chapter 3, Fig. 3.1B), in *N. brasiliensis* infection, relative gene

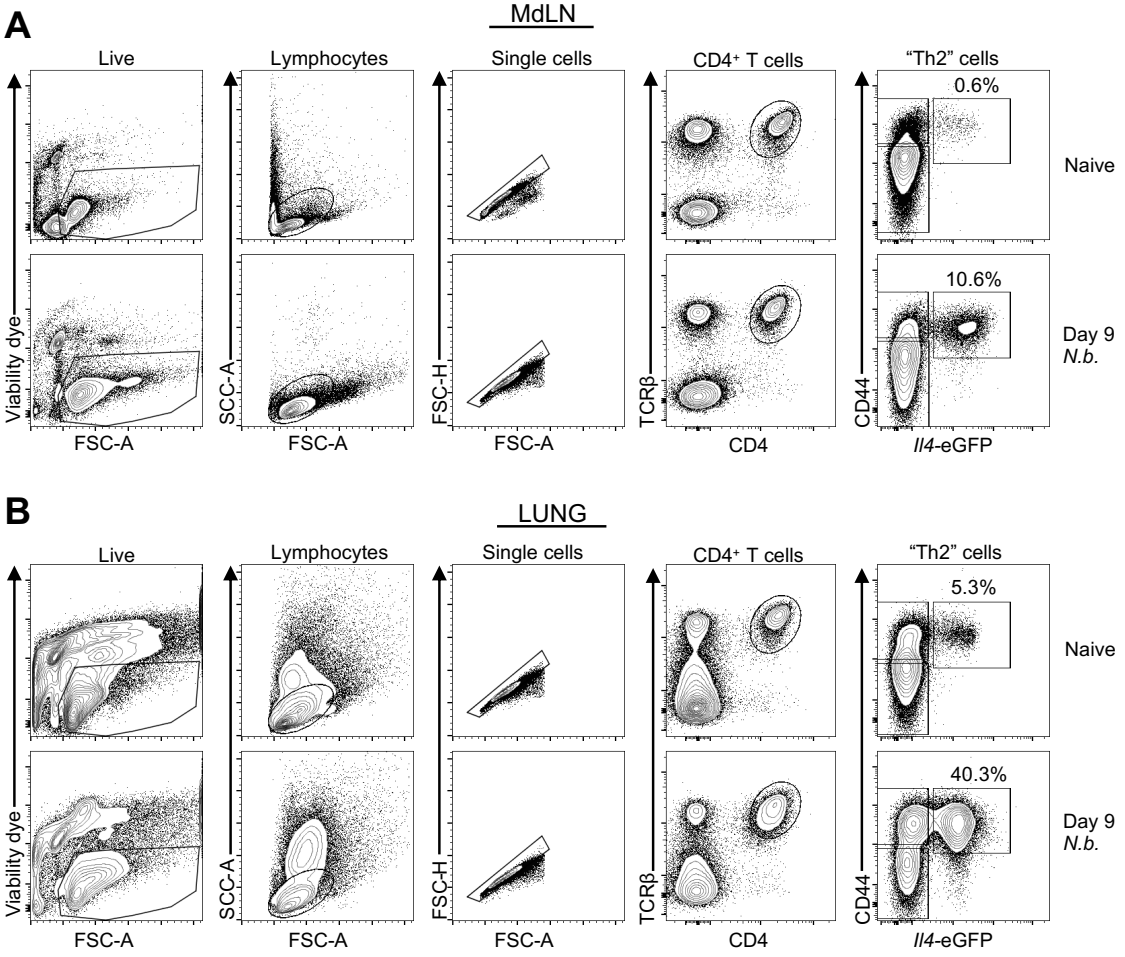

Figure 5.1 | Gating strategy for analysis during *N. brasiliensis* infection

The hookworm helminth, *N. brasiliensis,* was used to determine if there are metabolic changes in Th2 cells between the priming and tissues sites. B6.4get mice were infected with 250L3 larvae sub-cutaneously and the Th2 response was analyzed at 9 days post-infection. **A,** Representative staining in the mediastinal lymph node (MdLN). **B,** Representative staining in the lung. Uninfected mice were used as controls.

expression of key glycolytic enzymes showed a slight increase in activated Th2 cells in the MdLN compared to naïve CD4$^+$ T cells, particularly for *LDHA*, the final enzyme in the pathway (Fig. 5.2A). However, gene expression appeared to be stable in Th2 cells isolated from the lung compared to the MdLN, with no difference between *ENO1*, a slight but significant decrease in *PKM2*, and a slight but significant increase in *LDHA* (Fig. 5.2A). Interestingly, *HK2*, responsible for the first step in glycolysis, was the only gene to show a dramatic difference between tissues and was significantly upregulated in Th2 cells from the lung relative to those from the MdLN (Fig. 5.2A). Overall, however, this analysis suggests glycolysis does not experience further regulation at the genetic level in Th2 cells upon entry into the lung.

5.2.2 Tissue Th2 cells alter gene expression for FA metabolism

With the same sorted populations of naïve and Th2 cells analysed in Figure 5.2, I assessed the expression of genes pertaining to FA uptake and FAO. Relative gene expression of surface fatty acid transporters *FABP4* and *CD36* had a marked increase in Th2 cells isolated from the lung compared to those from the MdLN (Fig. 5.2B). The main transporter that shuttles fatty acids across the mitochondrial membrane for oxidation, *CPT1A*, also showed a significant increase in lung over MdLN Th2 cells (Fig. 5.2B). The transcription factor *PPARG* has previously been identified as a driver of Th2 function, and controls cellular fatty acid metabolism. As previously shown, *PPARG* expression is induced in Th2 cells after LN priming but is undetectable in naïve CD4$^+$ T cells (Fig. 5.2B). However, consistent with

expression of the FA transporters, *PPARG* is significantly up-regulated in Th2 cells residing in the lung compared to Th2 cells found in the MdLN (Fig. 5.2B). The stark induction of genes relating to FA uptake and FAO indicates that Th2 cells moving into the tissue could have an acute need for extracellular fatty acids.

Fatty acid synthesis (FAS) has been shown to increase upon T cell activation and is suggested to be a necessary for the activation process[143,211]. FAS is also regulated in a dichotomous fashion to FAO. I examined the gene expression of FAS rate-limiting enzymes to see if they were counter-regulated to support a metabolic programme of FAO in Th2 cells. In agreement with an increase in gene expression for fatty acid uptake, expression of fatty acid synthase (*FASN*) and acetyl-CoA carboxylase (*ACACA*) was significantly lower in lung Th2 compared to MdLN Th2 cells, while ATP citrate lyase (*ACLY*) showed no difference (Fig. 5.2C). Interestingly, expression of FAS enzymes in lung Th2 cells appeared comparable to that of naïve cells. In the MdLN, however, Th2 cells exhibited a significant increase in FAS gene expression compared to naïve cells, which supports the notion that FAS may be needed for initial T cell activation. Taken together, these data suggest that Th2 cells undergo a specific change in fatty acid metabolism after migration to the lung, favouring FAO while dampening FAS.

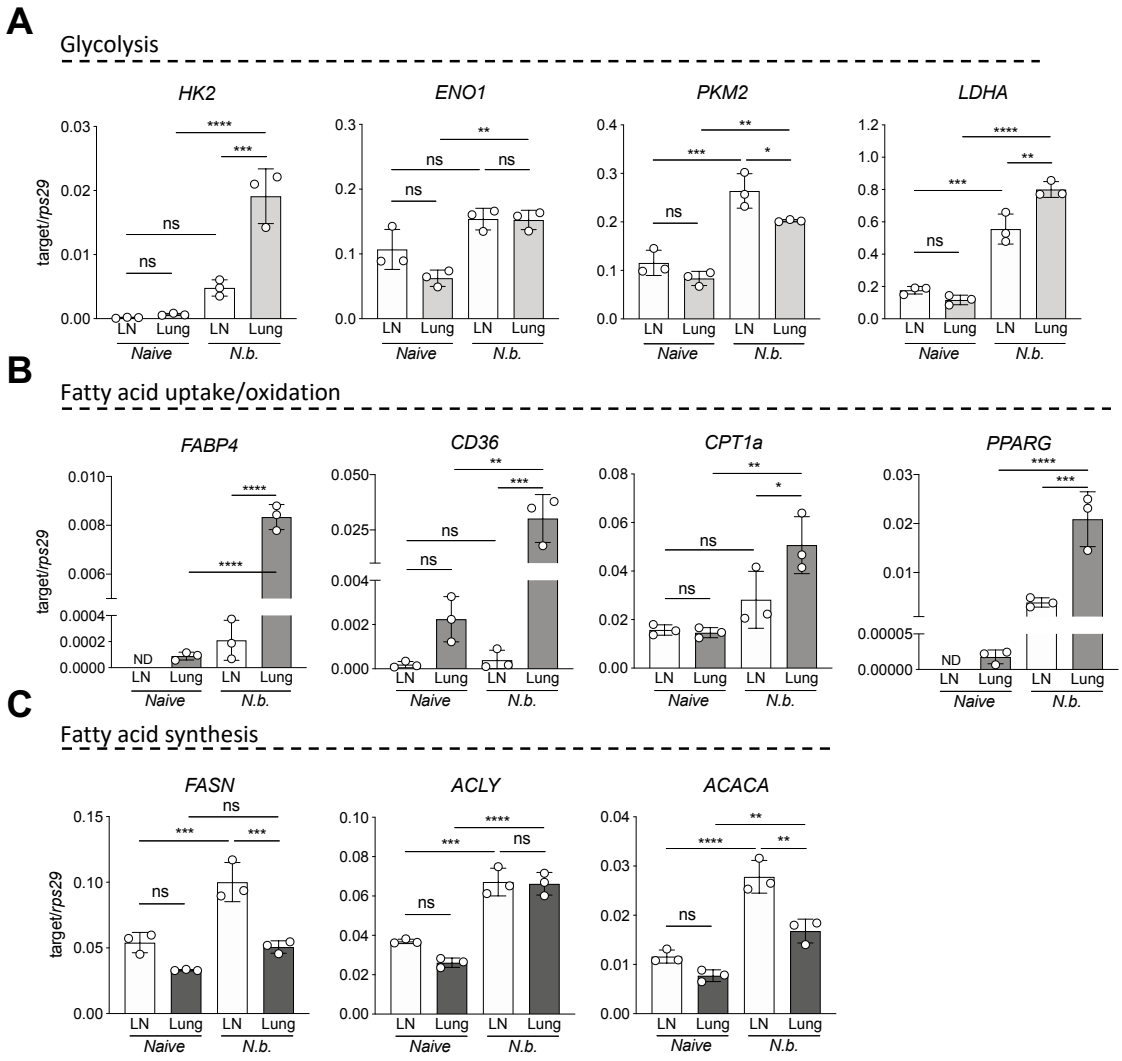

Figure 5.2 | Differential metabolic gene expression in MdLN and Lung Th2 cells

$CD44^{Hi}GFP^{+}$ Th2 cells were sort-purified from the MdLN and lung of *N.b.* infected B6.4get mice and analyzed for metabolic gene expression. $CD44^{Lo}GFP^{-}$ cells from uninfected mice were isolated in parallel to compare activated Th2 cells with naïve $CD4^{+}$ T cells. **A,** Gene expression for key enzymes in the glycolysis pathway. **B,** Expression of surface fatty acid transporters (*FABP4, CD36*), a mitochondrial fatty acid transporter (*CPT1a*), and transcriptional regulator of fatty acid uptake and oxidation (*PPARG)*. **C,** Gene expression of rate-limiting enzymes involved in fatty acid synthesis. Data representative of 2 independent experiments, n=3 per experiment, data points are samples pooled from 2 mice.

5.2.3 Comparable metabolic gene expression in Th2 cells between helminth infection and HDM challenge

To determine if low Th2 glycolysis and high gene expression for FAO is a consistent property of Th2 cells I used a non-helminth model of inflammation. B6.4get mice were sensitized with house dust-mite antigen (HDM) and challenged with the same antigen 10 days later, to simulate allergic asthma (Fig. 5.3A). Naïve $CD4^+$ and Th2 cells were flow-sorted for qPCR analysis of metabolic gene expression (Fig. 5.3A), however this experiment has only been done once.

Similar to *N. brasiliensis* infection, HDM activated Th2 cells in the MdLN demonstrated a small increase in glycolytic gene expression compared to naïve $CD4^+$ T cells (Fig. 5.3B). This increase was slightly more than was observed for Th2 cells from MLN during *H. polygyrus* infection (Fig. 3.3C), which may support the concept of glycolytic suppression by the parasite. However, the difference between naïve and activated Th2 cells from the MdLN during HDM challenge remains slight (Fig. 5.3B).

Also consistent with *N. brasiliensis* infection, Th2 cells from the lung of HDM challenged mice had comparable glycolytic gene expression to Th2 cells from the MdLN, further supporting that glycolysis is not upregulated in the effector tissue (Fig. 5.3B). HDM-induced Th2 cells also had significantly increased gene expression for *CD36* and *PPARG* in the lung relative to cells from the MdLN (Fig. 5.3C). Hence, low glycolysis may be common to multiple models of Th2 induction,

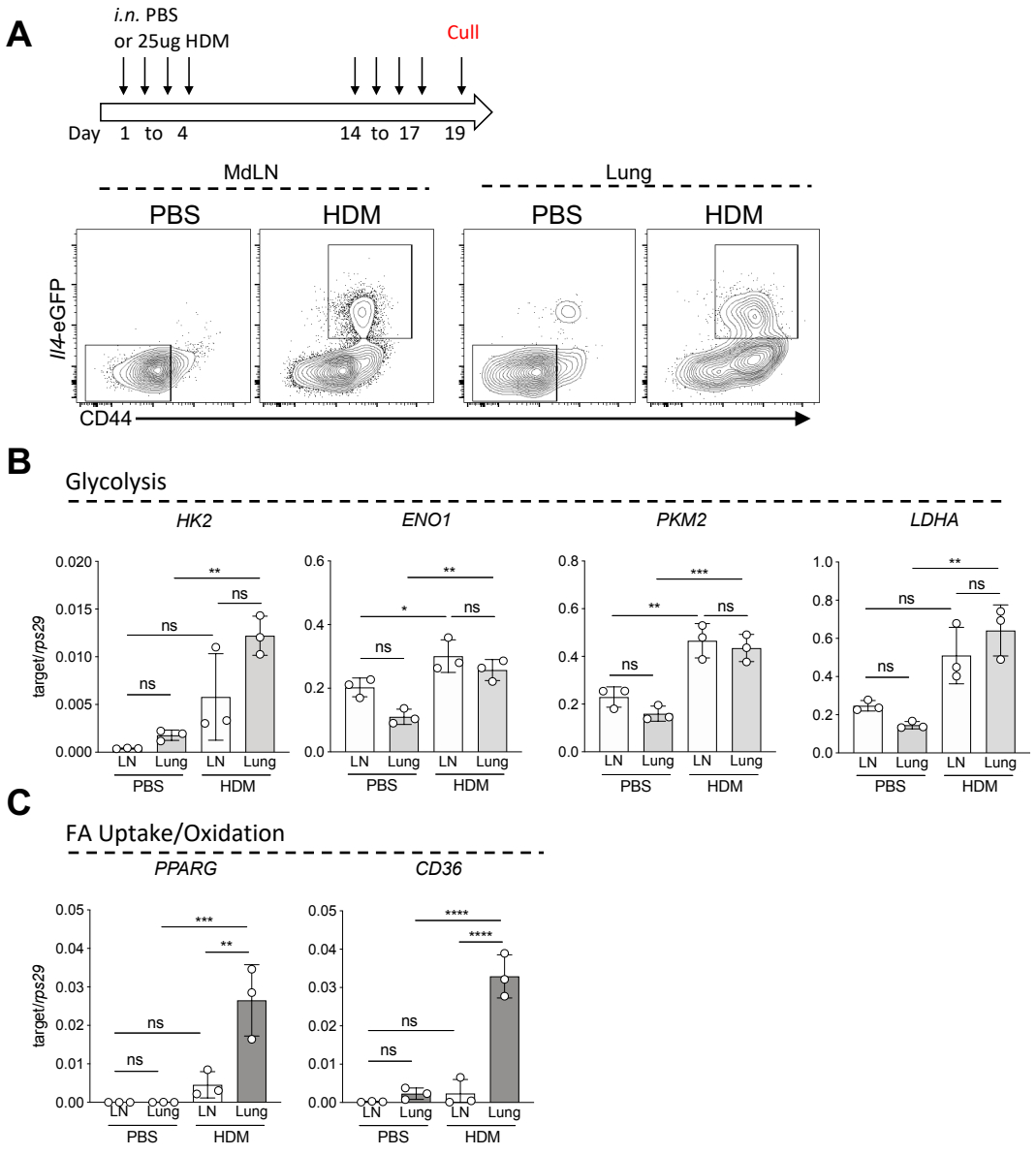

Figure 5.3 | Metabolic gene expression in Th2 cells after HDM challenge

A, Timeline for HDM sensitization and challenge, and representative sort plots for isolating HDM-challenge induced Th2 cells and naïve cells from PBS controls. **B,** Gene expression for glycolytic enzymes and **C,** expression of FA uptake and oxidation promoting genes in sorted HDM induced Th2 cells or PBS naïve $CD4^+$ controls. Data represents one experiment, n=3 mice per group.

and the same may be true for the lung-dependent expression of genes that promote FAO.

5.2.4 Increased FA uptake in ST2^{+} expressing cells is independent of IL-33 signalling

Given that Th2 cells purified from *N. brasiliensis* infected lungs displayed a marked increase in gene expression for FAO, I next investigated which signals could potentially be driving these metabolic changes. IL-33 is an important alarmin released within the lungs to induce cytokine production by Th2 cells. I therefore tested whether IL-33 had a role in altering FA uptake in cells taken from the lymph node. This was achieved by culturing sorted cells *ex vivo* overnight with or without IL-33, followed by brief culture with BODIPY FL C16, a fluorescent long-chain fatty acid (palmitate). For this experiment, to obtain the largest possible pool of lymph node Th2 cells, I isolated activated T cells from the MLN from *H. polygyrus* infected mice. The fluorescent emission of BODIPY FL C16 overlaps with that of GFP, and therefore, instead of sorting based on *il4* expression using B6.4get mice, cells were sorted according to CD44^{Hi} expression from C57BL/6 mice.

The impact of IL-33 on Th2 cytokine production was confirmed by determining the concentration of cytokines in culture supernatants. IL-33 signalling led to increased IL-5 and IL-13 secretion, but did not induce greater IL-4 expression, supporting the concept of temporal and spatial regulation between these cytokines (Fig. 5.4A). However, analysis of palmitate uptake revealed no difference between IL-33

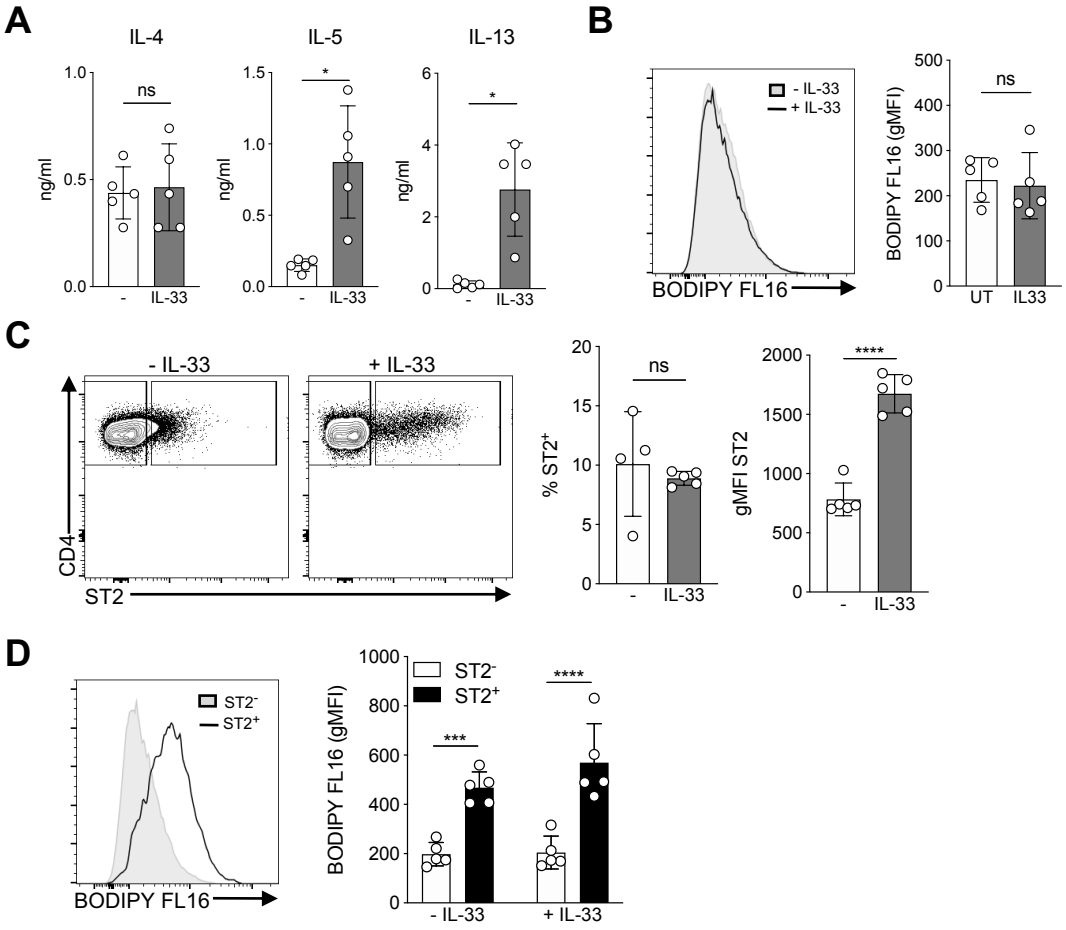

Figure 5.4 | IL-33 independent fatty acid uptake in *ex vivo* ST2$^+$ Th2 cells

CD4$^+$CD44^{Hi} cells were sorted from the MLN of day 14 *H. polygyrus* infected C56BL/6 mice, and re-stimulated *ex vivo* in the presence or absence of IL-33 cytokine for 18 hours. **A,** IL-33 induced changes in Th2 supernatant cytokines determined by cytometric bead array. **B,** Fatty acid uptake determined by flow cytometry in total CD4$^+$ cells using fluorescent palmitate, BOPIDPY FL16. **D,** Regulation of the IL-33 receptor, ST2, by IL-33 treatment. **C,** Fluorescent fatty acid uptake in CD4$^+$ populations segregated by ST2 expression in IL-33 treated and untreated re-stimulated cultures. Representative data of 3 experiments, n=6-10 mice pooled for cell sorting.

treated cells and those cultured in the absence of IL-33 (Fig. 5.4B). Therefore IL-33 does not appear to directly contribute directly to changes in Th2 FA metabolism.

To thoroughly confirm a lack of metabolic influence by IL-33, I selectively analysed the $CD4^+$ T cells responding to IL-33 by gating on those positive for the ST2 receptor. A similar frequency of $ST2^+$ cells was found between treatment conditions, indicating IL-33 exposure did not lead to an expansion of $ST2^+$ cells (Fig. 5.4C). However, IL-33 treatment increased the strength of ST2 expression on $ST2^+$ cells (Fig. 5.4C), fitting with previous studies that suggest IL-33 ligation positively regulates expression of its own receptor [279]. Interestingly, FA uptake was higher in $ST2^+$ cells compared to $ST2^-$ cells (Fig. 5.4D). Increased palmitate uptake was seen in the $ST2^+$ population from both IL-33 treated and untreated cultures (Fig. 5.4D). However, $ST2^+$ cells receiving IL-33 treatment did not increase FA uptake compared to $ST2^+$ cells without IL-33 (Fig. 5.4D). These data confirm that IL-33 elevates Th2 cytokine production independently of metabolic regulation, and further imply that IL-33 responsive cells selectively increase FA uptake prior to IL-33 exposure.

5.2.5 ST2 is expressed in lung but not lymph node Th2 cells

Recently, it has been proposed that PD-L1 expression on ILC2s facilitates adaptive Th2 responses to helminth infection via PD-1 signalling[83]. Studies on PD-1 have demonstrated it can act as a metabolic regulator for T cells[125,151,225,226]. In particular, engaging PD-1 with its ligands can promote lipolysis and FAO, causing

a shift towards mitochondrial metabolism while reducing glycolysis[125]. Of further interest, recent studies have reported a role for PPARγ in driving ST2 expression[231,287]. Combining these observations with my findings that *PPARG* expression is increased in Th2 cells from the lung, and that $ST2^+$ Th2 cells have a selective increase in FA uptake, I questioned if PD-1 alters Th2 cell metabolism after tissue migration into inflamed tissue, and subsequently promotes ST2 expression.

I first determined the expression patterns of PD-1 and ST2 in the MdLN and lung from *N. brasiliensis* infected mice. Infection led to a robust expansion of *il4*-$eGFP^+$ cells in both tissues, with the lung accumulating a much higher proportion of Th2 cells (Fig. 5.5A). Within the total $CD4^+TCR\beta^+$ population, PD-1 expression was significantly higher in the lung compared to either site from naïve mice, as well as the MdLN of infected mice (Fig. 5.5A). ST2 was also highly expressed by $CD4^+$ T cells in the lung, but not the MdLN of infected mice, nor in the MdLN or lung of naïve mice (Fig. 5.5A).

Next, I assessed the levels of PD-1 and ST2 expression specifically on *il4*-$eGFP^+$ Th2 cells, and on GFP^- non-Th2 cells separated into $CD44^{Lo}$ and $CD44^{Hi}$ populations. PD-1 expression was prevalent on Th2 cells, as well as on $CD44^{Hi}$ non-Th2 cells, from both the lymph node and lung, but not $CD44^{Lo}$ cells (Fig. 5.5B). Therefore, the increased frequency of PD-1^+ cells within the $CD4^+TCR\beta^+$ population in the lung was likely due to the larger proportion of activated Th2 cells.

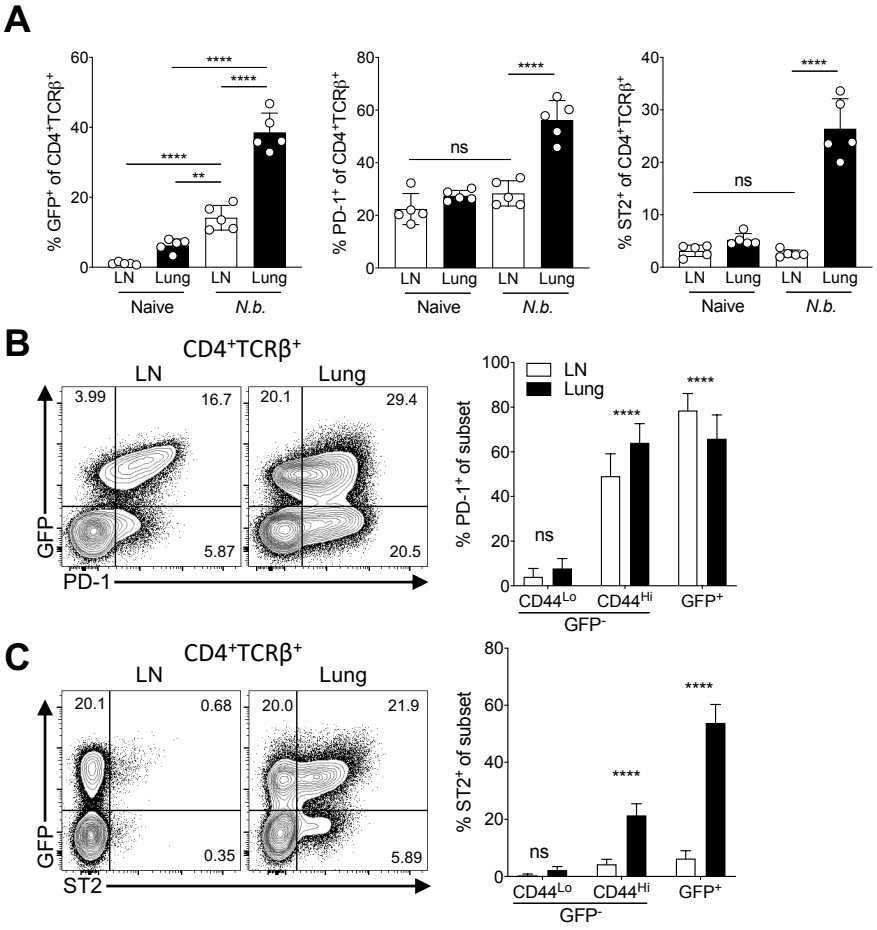

Figure 5.5 | ST2 expression is localized to the lung during infection

A, *il4*-eGFP, PD-1 and IL-33 receptor (ST2) expression in the MdLN and lung of naïve and day 9 *N. brasiliensis* infected B6.4get mice. **B**, Representative staining of PD-1 and **C**, ST2, against *il4*-eGFP expression in $CD4^{+}TCR\beta^{+}$ cells, and frequency of positive cells within the total GFP^{+} population, and within the $CD44^{Lo}$ and $CD44^{Hi}$ GFP- populations in the MdLN and lung of infected mice. **A** is representative of at least 3 experiments. **B** and **C** show 3 experiments pooled, n=5-7 mice per experiment.

In contrast, ST2 was nearly exclusive to the lung after gating on *il4*-expressing cells (Fig. 5.5C). Therefore, Th2 cells strongly express PD-1 following activation in the lymph node, but ST2 expression appears to be tissue-restricted.

5.2.6 ST2 is co-expressed with PD-1 on Th2 cells

To test the possibility that PD-1 could be a driver of ST2 expression in the lung, I assessed if both markers are co-expressed. Plotting PD-1 against ST2 on gated *il4*-eGFP^{+} cells revealed a distinct PD-1 and ST2 double-positive (DP) population found in the lung, but not the MdLN (Fig. 5.6A). This DP population equated to 40% of total Th2 cells (Fig. 5.6A). Conversely, Th2 cells in the MdLN consisted of more than 70% PD-1 single-positive cells, with double-negative (DN) cells forming the majority of the remainder (Fig. 5.6A). Amongst the *il4*-eGFP^{+} cells expressing ST2, up to 80% co-expressed PD-1, which was also true of the minor ST2^{+} population within the MdLN (Fig. 5.6B). A relationship between ST2 and PD-1 was further supported after dividing Th2 cells into 4 populations of increasing PD-1 expression and analysing the frequency and intensity of ST2 expression in each one, which demonstrated a strong positive correlation between both markers (Fig. 5.6C).

A small ST2^{+} population was evident in CD4^{+}TCRβ^{+} cells from the lung that did not express GFP (Fig. 5.5C and Fig. 5.6D). I analysed PD-1 and ST2 expression in this population to determine if a PD-1/ST2 axis may also exist in non-Th2 cells. Within the CD44^{Hi}*il4*-eGFP^{-} population, the frequency of ST2^{+} cells was reduced

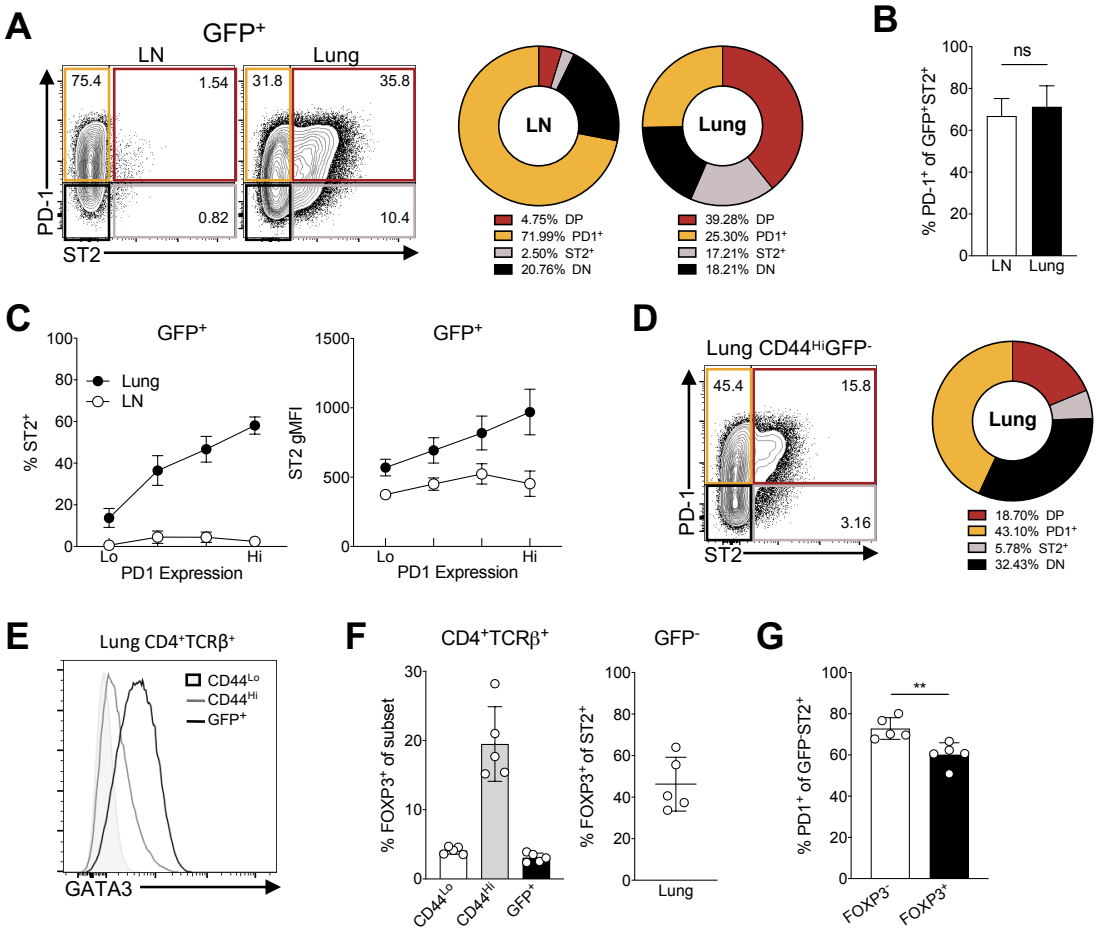

Figure 5.6 | ST2 expression strongly correlates with PD-1 in lung Th2 cells

A, Representative co-expression of PD-1 and ST2, gated using FMOs, in Th2 cells from the MdLN and lung, and mean frequency of PD-1 and ST2 negative (DN), single-positive and double-positive (DP) populations. **B**, Percentage of ST2$^+$ Th2 cells co-expressing PD-1 amongst *il4*-eGFP$^+$ lung Th2 cells. **C**, Correlation between frequency and intensity of Th2 cell ST2 expression with increasing PD-1. **D**, Representative co-expression of PD-1 and ST2 in activated, CD44^{Hi}GFP$^-$ cells from the lung, and mean frequencies of PD-1 vs ST2 populations. **E**, Representative GATA3 expression in Th2, activated non-Th2, and bystander populations in the lung. **F**, Abundance of Foxp3$^+$ regulatory T cells in the lung following *N. brasiliensis* infection, and frequency of GFP$^-$ST2$^+$ cells expressing Foxp3. **G**, Proportion of non-Th2 ST2$^+$ cells co-expressing PD-1 in Foxp3$^-$ and Foxp3$^+$ subsets. **A-D**, data from 3 experiments pooled, **E**, representative of 2 independent experiments, **F** and **G** represents one experiment. n=5-7 mice per experiment.

relative to Th2 cells, despite strong expression of PD-1 (Fig. 5.6D). Lesser ST2 expression was consistent with the observation that *il4*-eGFP^{-} cells had little GATA3 expression (Fig. 5.6E). However, the small ST2^{+} population in activated non-Th2 cells still mirrored the high level of co-expression with PD-1 (Fig. 5.6D).

In addition to Th2 cells, certain subsets of Tregs are known to express ST2[288,289]. By staining for Foxp3, I found that Tregs formed a large portion of activated, CD44^{Hi}*il4*-eGFP^{-} cells in the lung (Fig. 5.6F). This population of Tregs contributed half of the ST2^{+} population not expressing *il4* (Fig. 5.6F). After excluding Tregs from non-Th2 cells expressing ST2, PD-1 co-expression was similar to that of Th2 cells, of up to 80% (Fig. 5.6G). Foxp3^{+} Tregs expressing ST2, in comparison, had a slight reduction in PD-1 expression (Fig. 5.6G). However, Tregs have been shown to express ST2 prior to entry into the tissue[289].

Overall, these data show that ST2 expression is highly correlated with PD-1 on activated CD4^{+} T cells in the effector tissue during helminth infection. Therefore, my data thus far support a link between PD-1 signalling and the expression of ST2 after migration into the lung.

5.2.7 Activated T cells from the MdLN and lung have different rates of metabolite uptake

I have found that Th2 cells in the lung maintain low glucose metabolism but increase the uptake of FA, specifically in ST2^{+} cells. These metabolic traits are consistent with the consequences described downstream of PD-1 signalling[125]. To

test if there is connection between PD-1, metabolism, and ST2 expression, I isolated $CD4^+$ T cells *ex vivo* from *N. brasiliensis* infected C57BL/6 mice and cultured them briefly with fluorescent glucose (2NBDG) or palmitate (BODIPY FL C16).

$CD44^{Hi}$ cells isolated from either the MdLN or the lung rapidly acquired extracellular FA compared to $CD44^{Lo}$ cells (Fig. 5.7A). In spite of previous qPCR data indicating FA uptake may be enhanced in lung Th2 cells, no difference was detected between activated $CD4^+$ cells from the MdLN or the lung (Fig. 5.7A). However, $CD44^{Hi}PD\text{-}1^+$ cells had increased FA uptake in relation to $CD44^{Hi}PD\text{-}1^-$ cells (Fig. 5.7A). Further separation of cells based on PD-1 and ST2 expression showed that $PD\text{-}1^+ST2^+$ cells had significantly more palmitate uptake than single-positive or DN cells (Fig. 5.7A).

Dividing $CD44^{Hi}$ cells into populations of increasing PD-1 expression revealed that BODIPY FL C16 uptake positively correlated with PD-1 expression in both MdLN and lung $CD4^+CD44^{Hi}$ T cells (Fig. 5.7B). Though few cells were $ST2^+$ in the MdLN, ST2 expression increased slightly with an initial increase in PD-1, but then equally decreased as PD-1 expression continued to increase, while FA uptake increased linearly with PD-1 (Fig. 5.7B). Conversely, $CD4^+CD44^{Hi}$ T cells from the lung linearly increased both FA uptake and ST2 expression with increasing PD-1 expression to a strikingly similar degree (Fig. 5.7C). This suggests that, although FA uptake was not different between activated T cells between tissues,

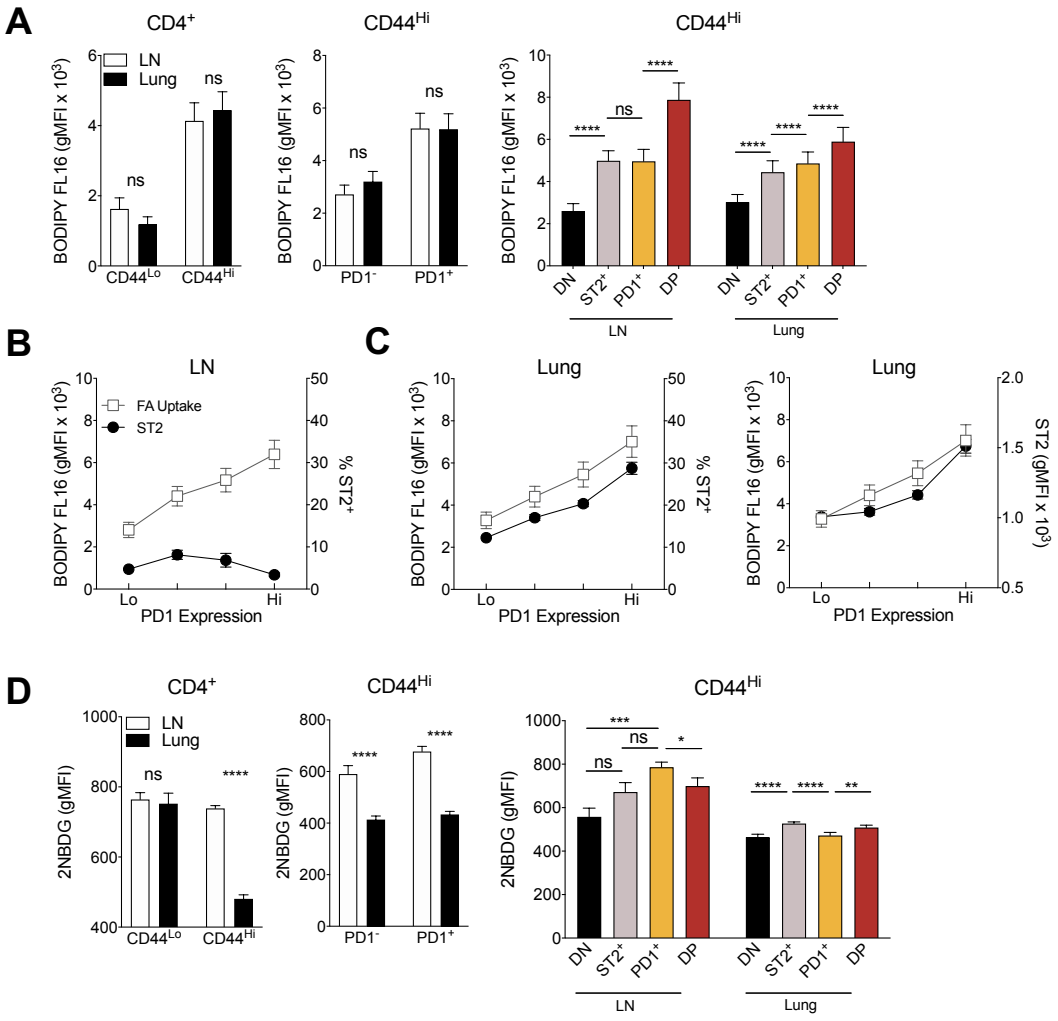

Figure 5.7 | Differential metabolite uptake in LN vs lung Th2 cells *ex vivo*

Metabolite uptake in cells from *N. brasiliensis* infection was determined by brief *ex vivo* culture of CD4$^+$ cells enriched by negative magnetic bead isolation from the LN and lung of C57BL/6 mice. **A**, Fluorescent BODIPY FL C16 (palmitate) uptake in activated and naïve CD4$^+$ populations, in activated total PD-1$^-$ and PD-1$^+$ cells, and in activated PD-1 and ST2 expressing subsets. **B**, Correlation between fatty acid uptake, PD-1, and ST2 expression in activated CD4$^+$ T cells from the LN and, **C**, from the lung. **D**, Fluorescent 2NBDG (glucose) uptake in activated and naïve CD4$^+$ cells, in total PD-1$^-$ and PD-1$^+$ populations, and in different ST2 and PD-1 expressing subsets. Data represent 3 independent experiments, with cells pooled from 4-6 mice.

ST2 expression strongly correlates with FA uptake in the lung. Furthermore, consistent with PD-1 driving a predominantly FA centric metabolism, PD-1^{+} cells had the greatest propensity to take up free FA in *ex vivo* culture.

In contrast, activated CD4^{+} T cells from the lung had significantly reduced glucose uptake compared to naïve or activated MdLN CD4^{+} T cells, or CD44^{Lo} cells also from the lung (Fig. 5.7D). PD-1 expression did not appear to correlate with reduced glucose uptake, as uptake was similarly low in PD-1^{-} and PD-1^{+} cells from the lung, and comparable in both populations in the MdLN (Fig. 5.7D). Glucose uptake was also consistently low in all of the different PD-1/ST2 expressing populations from the lung, though the minor differences observed were still statistically significant (Fig. 5.7D). Therefore, even though FA uptake is unchanged between locations, glucose uptake becomes restricted in activated CD4^{+} T cells following migration into the lung in response to *N. brasiliensis* infection. Despite no apparent role for PD-1 in glucose metabolism, cells with absent PD-1 expression may have expressed it before entering the lung. As over 70% of *il4*-eGFP^{+} cells in the LN were PD-1^{+}(Fig. 5.5B), I propose this is likely the case, and if true, low glucose uptake could potentially be attributed to PD-1 signalling.

5.2.8 Increased mitochondrial fitness in lung PD-1^{+} Th2 cells

A consequence of FAO is enhanced mitochondrial fitness[145]. To determine if FA being acquired by Th2 cells corresponds to changes in mitochondrial metabolism cells isolated from both sites were stained with dyes targeting the mitochondrial

membrane. Two different dyes were used to distinguish between total mitochondrial mass and mitochondrial potential (or activity), via flow cytometry.

I initially compared the mitochondrial properties of total Th2 cells by calculating the fold increase of fluorescent intensity in $CD44^{Hi}GFP^{+}$ cells relative to $CD44^{Hi}GFP^{-}$ cells as an internal control. This was done to offset the variability of staining between samples, and different tissues, due to the sensitive nature of the dyes. For both mitochondrial mass and potential, GFP^{+} Th2 cells from the lung showed a significantly greater fold-increase than GFP^{+} cells from the MdLN (Fig. 5.8A). To establish if PD-1 may be involved in altering mitochondrial metabolism, I then calculated the fold change of $PD\text{-}1^{+}GFP^{+}$ or $PD\text{-}1^{+}GFP^{-}$ cells relative to cells negative for both GFP and PD-1. Comparing these values illustrated that only GFP^{+} T cells from the lung acquired a notable increase in both mitochondrial mass and potential compared to double-negative cells, whereas MdLN GFP^{+} cells were similar to $PD\text{-}1^{-}GFP^{-}$ cells (Fig. 5.8B). Moreover, lung $PD\text{-}1^{+}GFP^{+}$ Th2 cells had the largest fold increase of any population, from either location, relative the GFP^{-} $PD\text{-}1^{-}$ cells (Fig. 5.8B). Lastly, both mitochondrial traits were highest in $PD\text{-}1^{+}ST2^{+}$ cells from both the MdLN and the lung (Fig. 5.8C and D).

These data further support that PD-1 expression on Th2 cells is driver of metabolic changes. Increased mitochondrial traits in $PD\text{-}1^{+}$ cells are also consistent with high FA consumption. The observation that activated MdLN $CD4^{+}$ T cells take up FA similar to those in the lung, paired with the differences observed regarding the

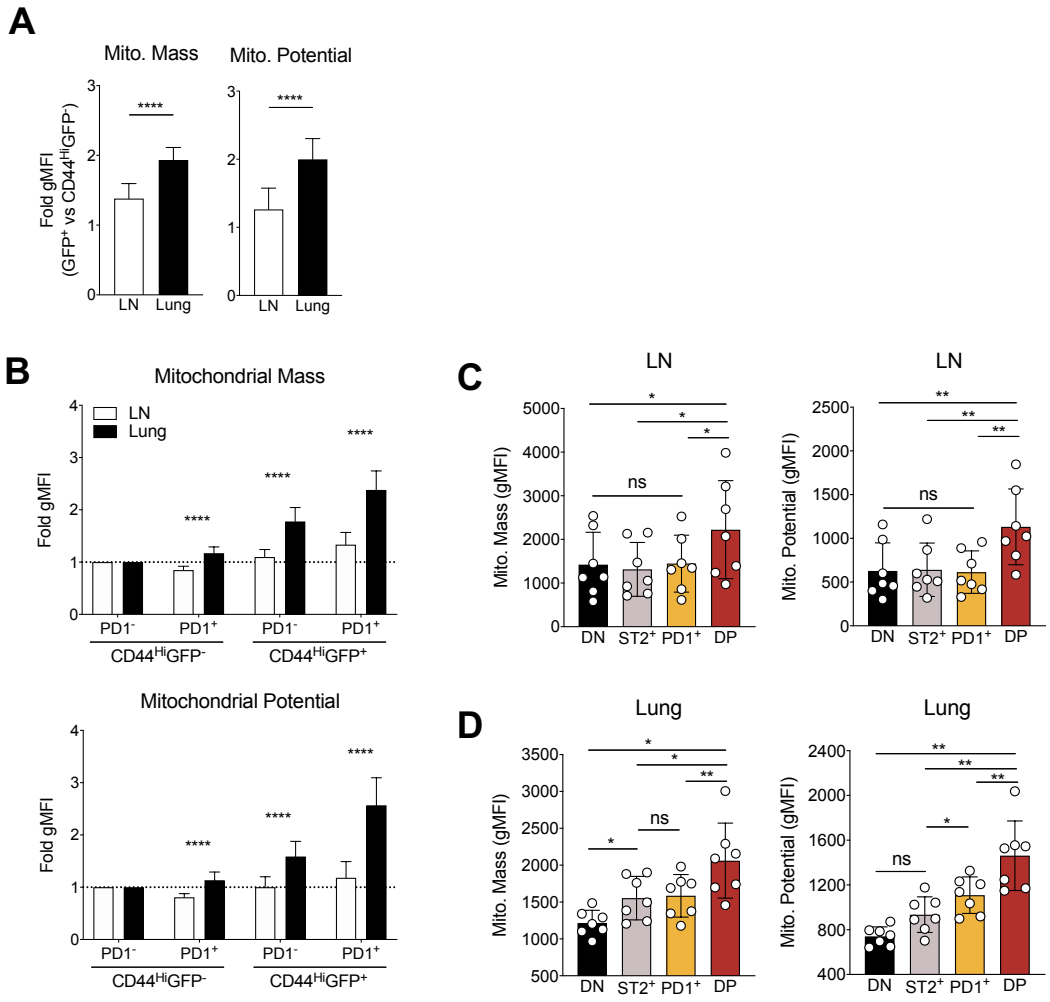

Figure 5.8 | Enhanced mitochondrial fitness in lung PD-1^{+} Th2 cells

MdLN and lungs were isolated from *N. brasiliensis* infected B6.4get mice and stained with Mitotracker Deep Red and MitoSpy Orange to assess mitochondrial mass and mitochondrial potential, respectively. **A**, Fold increase in fluorescent intensity of mitochondrial dyes in *il4*-eGFP^{+} Th2 cells relative to activated CD44^{Hi}GFP^{-} cells. **B**, Fold increase in intensity of mitochondrial staining for mass and potential in PD-1^{-} and PD-1^{+} populations within Th2 or activated GFP^{-} cells, relative to GFP^{-}PD-1^{-} cells. **C**, Mitochondrial mass in LN and lung PD-1 and ST2 expression subsets. **D**, Mitochondrial potential in PD-1 and ST2 expressing subsets from the LN and lung. **A** and **B** show data pooled from 3 experiments each with n=5-7 mice, **C** and **D** representative of 2 experiments with 7 mice per experiment.

mitochondria, may also indicate that Th2 cells from the different locations utilize environmental FA for distinct purposes.

5.2.9 High glucose uptake in the lymph node defines Tfh cells

Tfh cells are specialized producers of IL-4 in the draining lymph node during helminth infection[30], however, how Tfh metabolism differs from Th2 cells is unknown. In the lymph node, high PD-1 expression is used to help identify Tfh cells. Previously, I observed that *il-4* expression was highest in PD-1^{Hi} cells from the MdLN compared to lung during *N. brasiliensis* infection (Fig. 5.5B). Dividing CD4^{+}TCRβ^{+} cells into populations according to increasing PD-1 expression confirmed that the frequency of *il4*-eGFP^{+} cells in the MdLN increased with higher PD-1 expression (Fig. 5.9A). In contrast, CD4^{+} T cells from the lung reached an early plateau of *il4* expression (Fig. 5.9A). From these data, I predicted PD-1^{Hi} cells in the MdLN during *N. brasiliensis* infection were Tfh cells.

During my initial *ex vivo* characterization of glucose uptake I found that MdLN isolated T cells acquired more 2NBDG than cells harvested from the lung (Fig. 5.7D). Further stratification of CD44^{Hi} cells according to PD-1 showed that increasing PD-1 expression also corresponded to greater 2NBDG uptake in the MdLN (Fig. 5.9B). I therefore anticipated that high glucose uptake would be a feature of Tfh cell metabolism.

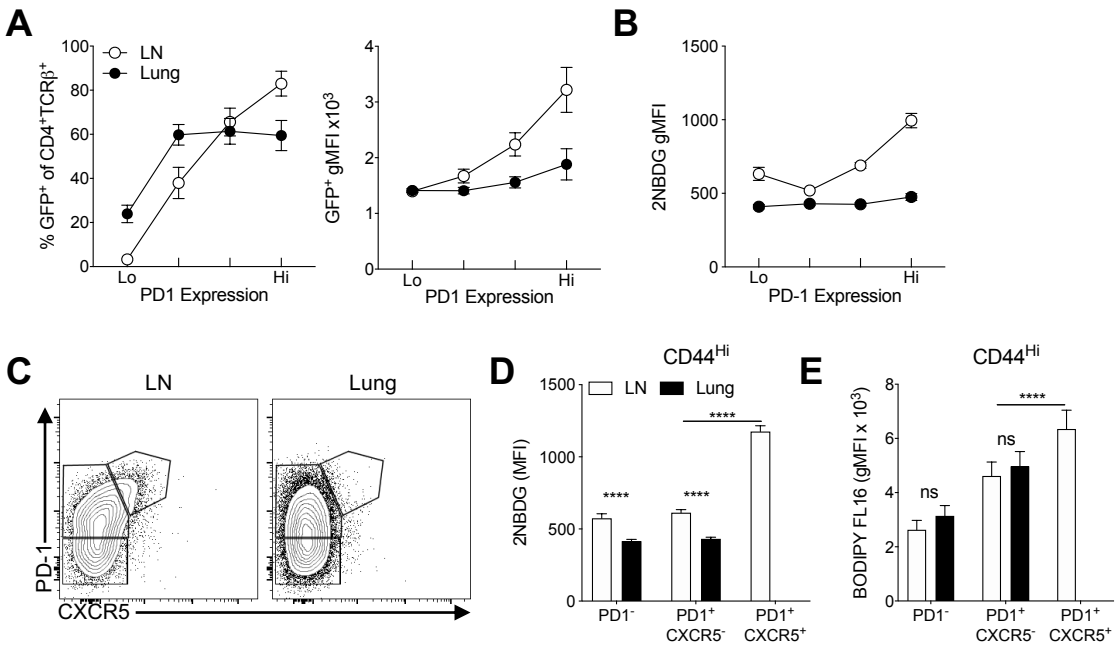

Figure 5.9 | High glucose uptake defines Tfh cells in the LN during *N. brasiliensis* infection

A, Relationship between the frequency and intensity of *il4*-eGFP expression with increasing PD-1 expression in MdLN and lung $CD4^+$ T cells from *N. brasiliensis* infection. **B**, Correlation of *ex vivo* uptake of fluorescent glucose analog 2NBDG with PD-1 expression. **C**, Representative staining for T follicular helper cells. **D**, Glucose uptake and **E**, fatty acid uptake, in LN Tfh cells and LN and lung $CD44^{Hi}$ non-Tfh populations based on PD-1 and CXCR5 expression. Data representative of 3 independent experiments.

Tfh cells were identified as PD-1^{Hi} cells co-expressing the germinal centre homing receptor, CXCR5. Accordingly, a PD-1^{Hi}CXCR5^{+} population was present in the MdLN and absent in the lung during *N. brasiliensis* infection (Fig. 5.9C). As predicted, these Tfh cells were enhanced in their ability to take up glucose compared to non-Tfh cells from the MdLN or from the lung (Fig. 5.9D). After brief culture with BODIPY FL C16, Tfh cells similarly demonstrated higher FA uptake over non-Tfh populations, though to a smaller extent than was observed for glucose uptake (Fig. 5.9E). These data indicate that Tfh cells are a highly metabolic subset compared to naïve CD4^{+} and Th2 cells during an ongoing Th2 response, and in particular, can be distinguished from Th2 cells in the MdLN according to their heightened propensity for glucose uptake.

5.2.10 Functional specialization of PD-1^{+}ST2^{+} lung Th2 cells

In the lung, I found that ST2 is co-expressed with PD-1 on Th2 cells, and cells that express both markers are quicker to acquire environmental FA relative to single- or double-negative Th2 cells. However, whether these findings are functionally relevant has yet to be shown. To assess the potential functional significance of joint PD-1 and ST2 expression, I determined the ability of different Th2 populations to produce cytokines. Similar to previous reports[43], intracellular staining of IL-5 and IL-13 following PMA and ionomycin stimulation demonstrated abundant cytokine expression from lung Th2 cells, whereas the MdLN had comparatively few cytokine-producing Th2 cells (Fig. 5.10A and B). PD-1^{+}ST2^{+} Th2 cells possessed the highest frequency of IL-5^{+} and IL-13^{+} cells and were more potent producers of

these cytokines (Fig. 5.10C, D). Additionally, performing the reverse analysis by gating on the total cytokine-producing population, for either IL-5 or IL-13, revealed that the vast majority of these cells are PD-1^{+}ST2^{+} (Fig. 5.10E). In agreement with PD-1^{+}ST2^{+} cells being the most functional Th2 cells, they also expressed the most GATA3 (Fig. 5.10F). Interestingly, *il4*-competent cells only expressing ST2 and not PD-1 had similar GATA3 expression to DP cells (Fig. 5.10F) but failed to express canonical Th2 cytokines to equivalent levels as the DP cells (Fig. 5.10C and D).

T cell effector function and metabolism are linked to proliferation, which can be restrained by PD-1 signalling[125,141]. To determine differences in proliferative ability between T cells from the MdLN and lung I stained for accessible nuclear Ki67. Activated *il4*-expressing cells from the MdLN had greater Ki67 staining than those from the lung, though no difference between tissues was observed in regard to GFP^{-}CD44^{Hi} cells (Fig. 5.10G). The increase observed in MdLN cells over the lung was limited specifically to PD-1^{+} cells, as no difference in Ki67 was observed between PD-1^{-} cells at the disparate sites while lung PD-1^{+} cells had significantly reduced Ki67 staining compared to the MdLN (Fig. 5.10G).

Altogether, my data suggest that PD-1^{+}ST2^{+} cells in the lung during *N. brasiliensis* infection, are functionally specialized to produce Th2 cytokines, and have reduced cell-cycle activity. These data may indicate that effector Th2 cells entering the tissue site are regulated to produce maximal cytokine, while simultaneously being restrained from further expansion.

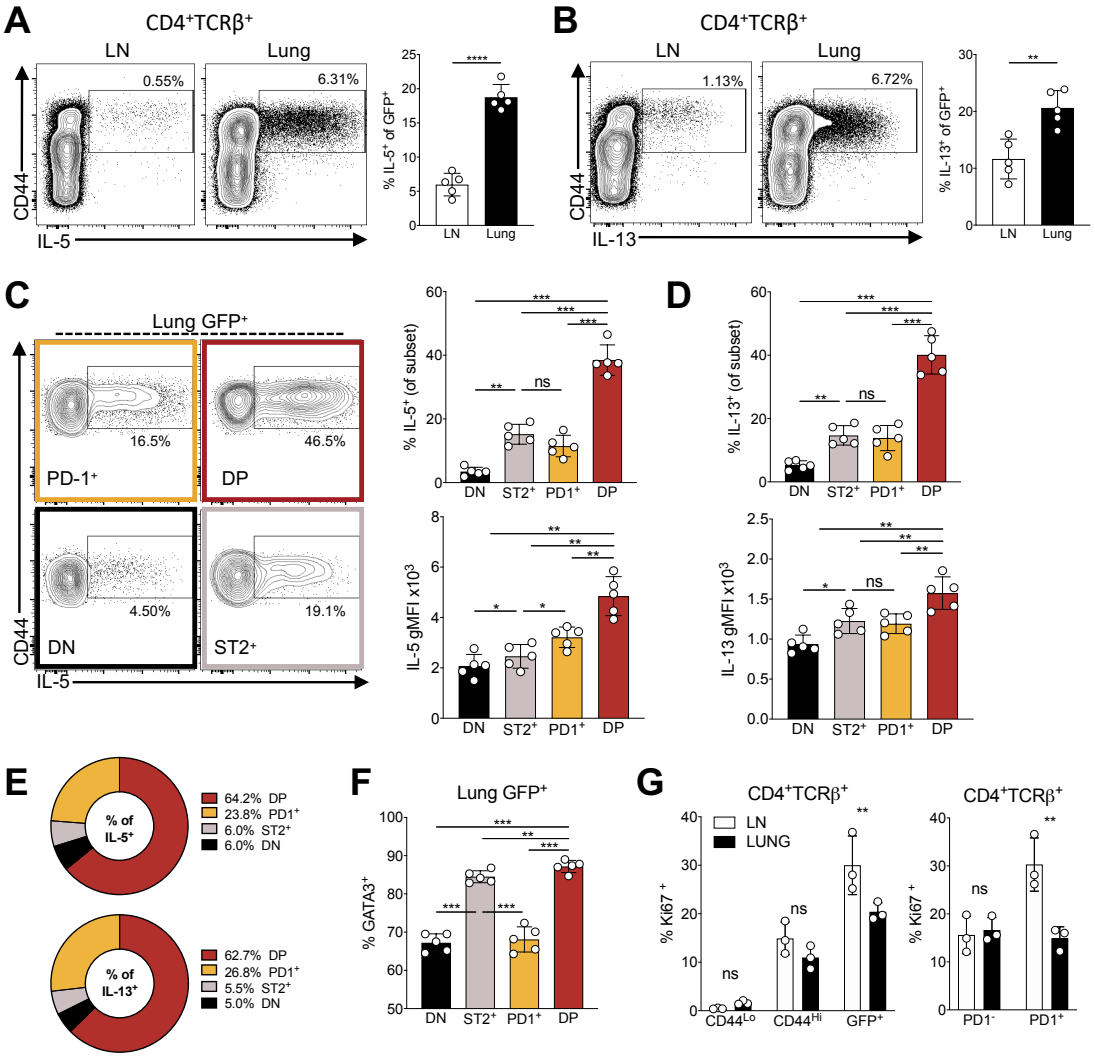

Figure 5.10 | Functional specialization of PD-1$^+$ST2$^+$ Th2 cells

A, Intracellular IL-5 and **B**, IL-13 expression following PMA/Ionomycin stimulation with monensin in the MdLN and lung of day 9 *N. brasiliensis* infected B6.4get mice. **C**, Representative staining, mean frequencies and gMFI of intracellular IL-5 expression from *il4*-eGFP$^+$ cells in PD-1/ST2 subsets. **D**, Mean frequency and gMFI of IL-13 expression from *il4*-eGFP$^+$ cells of PD-1/ST2 subsets. **E**, Distribution of PD-1 and ST2 expressing subsets within the total IL-5$^+$ or IL-13$^+$ populations. **F**, GATA3 staining in lung CD4$^+$ T cell populations, and prevalence of GATA3 expression within each *il4*-eGFP$^+$ PD-1/ST2 subset. **G**, Frequency of Ki67$^+$ cells in different CD4$^+$ T cells populations in the LN and lung. Data represent 2 independent experiments with n=3-7 mice.

5.2.11 PD-L1 promotes ST2 expression *ex vivo*

Others have recently shown that PD-1 signalling is able to promote lung Th2 responses during infection[83]. It has also been previously demonstrated that PPARγ, a regulator of FA metabolism, promotes Th2 responses through ST2 induction[287]. Furthermore, PD-1 ligation is able to promote FAO in T cells[125]. The rapid uptake of FA and co-expression of ST2 with PD-1 (Fig. 5.6 & 5.7) suggested PD-1 signalling may be a previously unidentified trigger for ST2 expression in Th2 cells.

To determine if PD-1 ligation could directly stimulate Th2 cells to express ST2, I sorted *il4*-competent $CD4^+TCR\beta^+$ T cells from the MdLN and the lung following *N. brasiliensis* infection and stimulated them with or without αCD3, in the presence or absence of purified agonistic PD-L1-Fc. After 3 days of *ex vivo* culture, I evaluated ST2 expression. Cells isolated from the MdLN had significantly more ST2 expression when re-stimulated with αCD3 and PD-L1 compared to αCD3 alone (Fig. 5.11A, C). The limited yield of cells from the MdLN was too small to test the effect of PD-L1 without αCD3. However, isolated lung Th2 without TCR stimulation cells displayed no differences in ST2 expression, whether or not the cells experienced PD-L1 ligation (Fig. 5.11B, C). With only αCD3, lung Th2 cells appeared to down-regulate ST2 expression from that of unstimulated cells (Fig. 5.11B, C). Excitingly, however, TCR stimulation in conjunction with PD-L1 caused a significant increase in the frequency and strength of ST2 expression compared to all other treatment groups (Fig. 5.11B, C).

Th2 cells re-stimulated with αCD3 adopted a prominent blasting phenotype, which was blocked by the addition of PD-L1-Fc (Fig. 5.11D). Consistent with PD-1 being a marker of recent activation, αCD3 stimulated cells strongly upregulated PD-1 expression (Fig. 5.11E). In contrast, the presence of PD-L1 maintained a PD-1^{Lo} population, suggesting a negative feedback loop between PD-1 signalling and its own expression. Th2 cells that remained PD-1^{Hi} after dual PD-L1 and αCD3 stimulation also displayed the strongest ST2 expression, further supporting ST2 as a target of downstream PD-1 signalling (Fig. 5.11E). Th2 cells that were PD-1^{Lo} expressed ST2, but significantly less than PD-1^{Hi} cells. A similar PD-$1^{Lo}ST2^{+}$ population was observed in Th2 cells left unstimulated following cell-sorting (Fig. 5.11E). Therefore, this expression is likely preserved from the time of isolation.

As sorted T cells were originally purified using *il4*-eGFP, I looked at whether *il4* expression persisted *ex vivo* to gauge if PD-1 signalling impacted cytokine expression. Without αCD3 stimulation, GFP expression was lost, indicating cells stopped producing *il4* transcript once in culture (Fig. 5.11F). Secondary activation by αCD3 induced a dramatic increase in *il4* expression, which was completely blocked by the presence of PD-L1-Fc. Hence, although PD-1 induces ST2 expression, in the absence of exogenous IL-33, PD-1 may inhibit cytokine production.

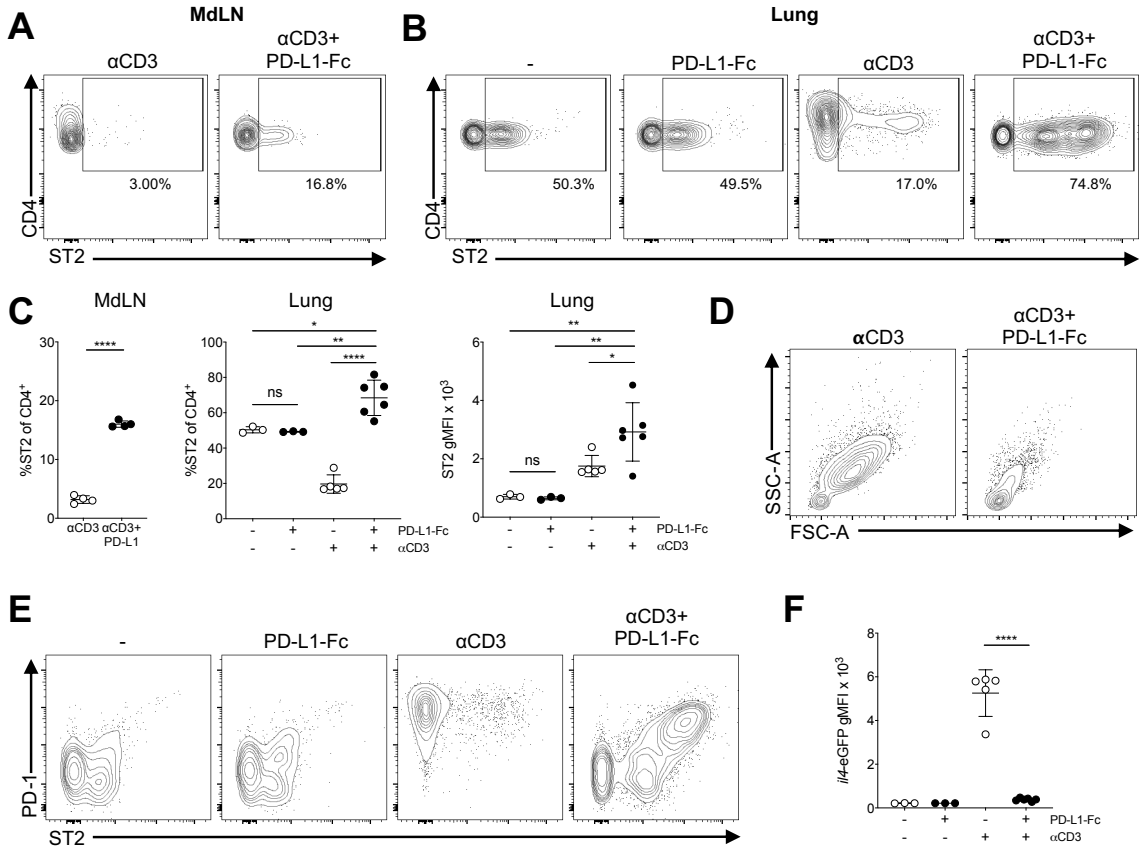

Figure 5.11 | PD-L1 directly promotes ST2 expression

Representative flow cytometry plots of ST2 expression of *il4*-eGFP^{+} cells sorted from the **A**, MdLN and **B**, lung of day 9 infected mice and re-stimulated with 1 µg/ml αCD3 or 5 µg/ml plate bound PD-L1-Fc for 3 days. **C**, Frequency of ST2^{+} cells of re-stimulated MdLN, and frequency and gMFI of re-stimulated lung Th2 cells. **D**, Blasting profile of re-stimulated Th2 cells sorted from the lung. **E**, Representative co-expression plots of PD-1 and ST2 in re-stimulated lung Th2 cells. **F**, Expression of lung Th2 *il4*-eGFP after 3 days of re-stimulation. One of 3 independent experiments shown. Data points are technical replicates from pooled samples of 7-9 mice.

In conclusion, these data provide evidence for a model in which Th2 cells encounter tissue-localized PD-1 signalling alongside secondary TCR stimulation to promote IL-33 responsiveness, through regulation of the receptor ST2. The ability of Th2 cells to respond to IL-33, following PD-1 stimulation, aligns with the finding that PD-1^{+}ST2^{+} cells are the most potent cytokine-producing CD4^{+} T cells in the lung (Fig. 5.10). Interestingly, PD-1 expression corresponded to reduced proliferation and may contribute to contraction of the effector response, while still allowing cells to produce cytokines. FA uptake and mitochondrial properties (Fig. 5.7 & 5.8), on the other hand, were increased with PD-1 expression. From this observation, I have further hypothesized that this increase in FA metabolism may be a mechanistic link between PD-1 signalling and ST2 expression. Current work is ongoing to test this hypothesis.

5.3 Discussion

I have shown that Th2 glycolysis remains similar to, or even reduced, compared to naïve CD4^{+} T cells after initial priming during helminth infection, dispelling the current paradigm that Th2 are highly glycolytic[121,191,192,202]. This prompted two questions regarding the regulation of Th2 metabolism. First, is glycolysis driven by tissue-restricted signals; and second, are other metabolic pathways supportive of Th2 cell function?

In this chapter, I indeed found that a metabolic checkpoint for Th2 cells existed in the tissue, but fatty acid uptake, rather than glycolysis was increased. *Ex vivo* measurement of glucose uptake demonstrated a decrease in lung Th2 cells compared to naïve $CD4^+$ T cells. This metabolic shift was in accordance with published effects of PD-1 signalling[125,151,226] and was observed to occur preferentially in $ST2^+$ cells, independent of IL-33. From these findings I proposed a model in which Th2 metabolism, tailored by PD-1 ligation, permits localized cytokine secretion in the effector tissue via ST2 induction. In support of this model, PD-1 directly increased expression of the ST2 receptor in Th2 cells *ex vivo*. Therefore, by combining metabolic phenotyping with results published by others, I have identified a novel regulatory mechanism for Th2 function in the effector tissue.

Research from other groups has previously provided circumstantial evidence that fatty acid uptake and oxidation may be a feature of Th2 metabolism. Namely, multiple studies have assigned a critical role for PPARs, master regulators of FA metabolism, in Th2 function[147,231,287,290,291]. PPARγ in particular is expressed in Th2 and not Th1 or Th17 cells, and CD4-specific deletion of PPARγ impairs resistance to helminth infection and reduces pathology in allergic challenge[231,287]. In line with my work, PPARγ deficiency specifically impaired ST2 expression and peripheral Th2 cell responses[287]. However, here I have confirmed for the first time that Th2 cells generated *in vivo* rapidly take up fatty acids compared to glucose.

It is still unknown whether or not the activity of PPARγ in Th2 cells contributes to changes in FA metabolism, and if metabolic changes are functionally necessary. Th2-licensing dendritic cells[231], alternatively activated macrophages[166], and ILC2[292] all highly express PPARγ. Interestingly, IL-4-induced STAT6 activation in macrophages directly interacts with PPARγ to promote gene expression of targets needed for FA uptake and oxidation[293]. IL-4-stimulated macrophages lacking PPARγ have a reduced ability to import and oxidize FA and have reduced mitochondrial content compared to WT macrophages[166]. Similarly, I found increased FA uptake and mitochondrial properties in Th2 cells that highly express PPARγ. Directly blocking FA uptake or oxidation in macrophages or ILC2 has further been shown to prevent their polarization or expansion[132,285]. Therefore, PPARγ induced FAO has a significant functional role in type 2 immunity, and with evidence from my work, likely Th2 function.

It has already been established that PPARγ can support Th2 function in the tissue by promoting ST2 expression[287], but the stimulus responsible for PPARγ expression has not yet been identified. IL-4 can directly induce PPARγ expression and regulate PPARγ activity in macrophages and dendritic cells[166,293]. As Th2 cells isolated from the lymph node, a concentrated site for IL-4 production[294], have significantly less PPARγ expression compared to Th2 cells from the lung, additional signals must be needed.

Metabolic consequences have been described for PD-L1 ligation of PD-1 on T cells[125,151,226]. The presence of PD-L1 at the time of, or following, primary $CD4^+$ T cell activation reduced glucose metabolism and fatty acid synthesis compared to cells activated normally[125]. PD-L1 treatment simultaneously elevated FA uptake and FAO. The oxygen consumption rate, indicative of mitochondrial metabolism, was also reduced with PD-L1. Yet, these cells maintained a high spare respiratory capacity[125], suggesting they can dynamically regulate mitochondrial activity in times of stress or a changing environment[144]. Similar metabolic attributes have been discovered when comparing $CD8^+$ T cells of varying PD-1 expression during chronic viral infection[151]. In this study, PD-1 sufficient and *Pdcd1*$^{-/-}$ T cells that lack PD-1 were transferred into the same recipient, and following LCMV infection, *Pdcd1*$^{-/-}$ T cells had higher glucose uptake and reduced mitochondrial mass. Therefore, the reduced glucose uptake, the increased procurement of FA, and the increased mitochondrial mass presented by tissue-Th2 cells are all consistent aspects of PD-1 signalling, as well as immune cell PPARγ activation[293]. PD-1 triggering could therefore be an upstream director of PPARγ expression and activity.

It has recently been reported that PD-L1 expression on ILC2 in the lung is needed to maximize the adaptive Th2 response to *N. brasiliensis*[83]. Separate research using *Trichinella spiralis* also demonstrated that PD-1 promotes Th2 cytokine production during helminth infection[295]. PD-L2 has furthermore been shown to exacerbate asthma in mice[296]. However, these studies are at odds with earlier

studies suggesting that PD-1 is inhibitory of Th2 function. Neutralization of PD-1 or its ligands during infection with different helminth models, including *Schistosomiasis*[297]*, Litomosoides sigmodontis*[261], as well as *N. brasiliensis*[298], has been associated with improved Th2 expansion and cytokine production.

The discrepancy in roles for PD-1 on Th2 cells can likely be attributed to the time and location dependent context of PD-1 ligand expression. Inhibitory PD-1 signalling in Th2 cells is most strongly associated with PD-L2 expression on AAM[261,298]. However, PD-L1 appears to be specifically required for the transition to effector Th2 cells in the tissue[83]. In the lung, alveolar macrophages have displayed insensitivity to IL-4 and fail to adopt an AAM phenotype[299], suggesting they may also not express PD-1 ligands, for instance in the context of *N. brasiliensis* infection. Moreover, earlier approaches used to determine the role of PD-1, such as systemic antibody blockade or global deficiency, would have mitigated any temporal intricacies in signalling. In addition, T cells were commonly assessed from secondary lymphoid organs, thus PD-1 ligation in the draining lymph node may have distinct outcomes compared to when it occurs in the tissue.

In the lymph node during an ongoing immune response, high PD-1 expression is a defining marker of Tfh cells. However, PD-1 signalling in Tfh cells, originating from PD-L1 on DCs or B cells, restrains Tfh cell expansion[223,300–302]. PD-L1 deficiency can hence lead to an overgrowth of potentially auto-reactive Tfh cells[223]. Consistent with a PD-1 driven metabolic programme, I found that Tfh cells take up

large amounts of environmental FA, to a greater extent than naïve cells or activated non-Tfh cells. Although genes pertaining to FA uptake and oxidation had increased expression in *il4*-expressing cells from the lung compared to MdLN, cells from either tissue acquired FA to a similar degree. Despite this disparity in data, I found that lung Th2 had increased mitochondrial properties that suggest the use of FA is biased toward FAO in the lung compared to the LN. Interestingly, Ki67 staining revealed MdLN cells expressing *il4*-eGFP, many of which are likely Tfh cells, had better proliferative potential. Therefore, extracellular FA could be supplying the growth of cellular and organelle membranes to support rapid Tfh cell division, as opposed to fuelling mitochondrial OXPHOS.

Though PD-1 signalling has been demonstrated in Tfh cells, and Tfh have a high capacity to take up FA, they do not express ST2. GATA3 is required for sustained ST2 expression in T cells[280]. However, forced expression of the Tfh lineage transcription factor BCL-6 hinders both GATA3 expression, and subsequent Th2 differentiation[34,35,303]. Thus, the lack of expression of ST2 on Tfh cells might be explained by the antagonistic behaviour of GATA3 and BCL-6. The use of an IL-21 reporter to identify Tfh cells in immunized mice has previously confirmed that Tfh cells do not express GATA3 beyond naïve levels[304]. Hence, though Tfh receive PD-1 signals, it is unlikely they possess the correct transcriptional machinery for ST2 expression.

How expression of ST2 is regulated is generally not well understood, however. Here, I have demonstrated that tissue-specific ST2 expression can be promoted by PD-1. Interestingly, naïve $CD4^+$ cells after primary activation *in vitro* under Th2-polarizing conditions do not express ST2[305]. Hence, despite a requirement for GATA3, signals other than canonical cytokines are needed for transcription of *il1rl1*, the gene encoding ST2[306]. Multiple rounds of stimulation are sufficient to force ST2 expression, with increasing expression after each subsequent stimulation[305]. An interesting question then, is whether ST2 is linked to T cell exhaustion or anergy, which is thought to occur in response to repetitive antigen exposure. These cellular states have similarly been linked to a switch from glucose metabolism to FAO[151,153]. Incidentally, $CD4^+$ T cells that seed peripheral tissues early after birth have been shown to highly express ST2, and in this context ST2 deficiency accelerated an anergic phenotype, while exogenous IL-33 led to a breakdown in tolerance[307]. Intriguingly, these $ST2^+$ cells were also only found within a $PD\text{-}1^+$ population. Thus, although our knowledge is limited regarding how ST2 expression is induced, there is an emerging pattern that may hint at a link between PD-1, anergy/exhaustion, and possibly FAO.

From a molecular perspective, our understanding of the mechanisms needed to authorize *il1rl1* transcription is similarly poor. There is now abundant evidence illustrating the necessity of PPARγ for ST2 expression. PPARγ deficiency leads to a reduction in ST2 gene and surface expression, and agonistic ligands for PPARγ induce ST2 *in vitro*[231,287]. Yet studies thus far have failed to prove whether PPARγ

can directly bind or regulate the *il1rl1* locus[287]. The distinct expression pattern of ST2 between lymphoid and peripheral tissue Th2 cells indicates that epigenetic regulation could be a factor. Accordingly, it has been shown that accessibility of the *il1rl1* gene is determined by activity of the glycolytic enzyme, pyruvate kinase M2 (PKM2), during ILC2 development[308]. Enhanced glycolysis, achieved by constitutively activating HIF-1α, impaired ILC2 development by downregulating ST2, but development could be restored by blocking glycolysis or PKM2. This may also explain why ST2 expression is restricted in the lymph node, where activated T cells consume more glucose than cells in the lung. A permissive *il1rl1* locus in ILC2s was associated with increased histone methylation[308]. Coincidentally, it has been shown that glycolysis promotes IFNγ gene expression by providing acetate for histone acetylation, as opposed to methylation[195], providing further juxtaposition between metabolism in type 1 and type 2 responses. Overall, a connection has been made between cellular metabolism and ST2 expression via an epigenetic mechanism. PPARγ may have an important role in aligning cellular metabolism so that it is conducive to the correct epigenetic modifications of the *il1rl1* gene locus.

PD-1 is a well-established negative regulator of T cell effector function, including proliferation and cytokine production, during TCR mediated activation[213]. When culturing Th2 cells *ex vivo* with PD-L1, I found that ST2 was only induced when the cells also received TCR stimulation. This could also be true *in vivo*, as tissue-resident ILC2 can interact with T cells via PD-L1, and are also able to activate Th2

cells through the presentation of peptide-MHCII[82,83]. In accordance with the suppressive nature of PD-1 signalling, PD-L1 treated Th2 cells displayed a reduced blasting profile compared to those only receiving TCR stimulation. *In vivo, il4*-eGFP$^+$ cells in the lung elicited by *N. brasiliensis* infection had less Ki67 staining than those isolated from the MdLN, suggesting they have a decreased ability to proliferate; potentially a consequence of PD-1 ligation. As discussed previously, PD-1 may also limit the excessive proliferation of activated cells in the lymph node, mainly Tfh cells, yet this inhibitory effect may be offset by environmental factors, such as hypoxia[309].

Altogether, data from this chapter imply that Th2 cells need secondary activation in the tissue, while PD-1 restrains aberrant expansion of cytokine producing effector cells. In other words, PD-1 may serve a dual purpose to permit localized cytokine secretion via ST2 expression, while limiting the size of the effector pool to reduce the risk of a pathological response. Interestingly, despite the observation that PD-1 signalling promotes ST2 expression, it simultaneously abrogated *il4* expression. PD-1 signalling might also then restrain Th2 function until it receives sufficient signals (i.e. IL-33) to secrete cytokine again. Accordingly, in mice lacking the ST2 receptor, CD4$^+$ IL-4 expression in the lung but not the MdLN was decreased compared to WT mice[43]. Hence, the addition of IL-33 may reinvigorate IL-4 production as well as inducing IL-5 and IL-13.

Chapter 6: Final Discussion and Conclusions

Th2 cells, rarely discussed within the field of immunometabolism, are cited as highly glycolytic cells, and even as the most glycolytic $CD4^+$ Teff subset[121,175–177,191,192,202]. While *in vitro* studies argue in favour of this, the real metabolic phenotype of Th2 cells *in vivo* has yet to be published. Transgenic mouse models and *in vitro* studies cannot fully tease apart whether metabolism is directly impacting the cell, is a bystander consequence of signalling, or whether interventions are targeted specifically to the pathway of interest. What metabolism looks like during a "normal" *in vivo* response still needs to be defined. Therefore, the first aim of this thesis was to determine an *in vivo* metabolic phenotype of Th2 cells, during helminth infection.

6.1 Glycolysis as a requisite for Th2 differentiation

In Chapter 3, I showed that Th2 cells isolated from the lymph node, during active *H. polygyrus* infection, used glycolysis at a more comparable degree to naïve $CD4^+$ T cells than *in vitro* activated cells, or Th1 cells isolated from *T. gondii* infection (Fig.3.3-3.6). This raised a question regarding the necessity of glycolysis for Th2 cell function *in vivo*. Several earlier reports suggest glycolysis is a requirement for Th2 cells, as removing mTOR components in a CD4-specific manner blocked Th2 development during HDM challenge[191,192]. The major repercussion of mTOR deficiency, however, was that cells remained in the G_0

phase of the cell-cycle, and therefore did not fully activate or proliferate[192]. In this study, the authors attempted to segregate activation from function by showing that, *in vitro*, weak inhibition of glycolysis augmented IL-4 production without impacting cell division. Interestingly, the late addition of 2DG to cultures had no effect on IL-4 production, which suggests glycolysis may not be required for cytokine production once the cell is fully activated. In contrast, my data showed that activated, *il4*-expressing $CD4^+$ T cells from the MLN of infected mice produced less cytokine after *ex vivo* restimulation with 2DG present, suggesting that the minor increase in glycolysis from the naïve state is functionally important (Fig. 3.9).

To reconcile the data discussed above, caveats from both systems must be addressed. As activated T cells grow, they increase their metabolic machinery and activity. Hence, previously activated cells may need to be treated with higher concentration of 2DG to achieve the same inhibitory effect as seen with naïve cells treated at the time of activation. Conversely, multi-day *ex vivo* stimulation via the TCR will have metabolic consequences and force unphysiological levels of cytokine production that may be more affected by 2DG inhibition than would normally be observed in cells stimulated by cognate TCR antigen *in vivo*. To formally determine if glycolysis is needed for Th2 cytokine production, I would directly stimulate and intracellularly stain sorted Th2 cells *ex vivo* from infection in the presence or absence of glucose. This could be supported by an *in vitro* system where Th2 cells are cultured normally before being transferred into control or glucose-free media for stimulation and cytokine detection. Modulating glucose in

the media as opposed to using 2DG to block glycolysis means that differences in cytokine production can be attributed more directly to glucose metabolism, and not to possible off-target effects of the inhibitor. If glycolysis had a direct role in permitting cytokine production, these comparisons will show reduced cytokine production in the absence of glucose. If no difference is observed, it can be concluded that glucose metabolism supports differentiation, but not effector function.

However, the importance of glycolysis may vary depending on the source of Th2 cells. Namely, Th2 cells in the lymph node behave distinctly from those in the effector tissue[43]. The fact that my initial metabolic characterization was done using cells isolated from the MLN is something that needs to be kept in consideration. In Chapter 5 I therefore examined Th2 cells from different sites. Between the MdLN and the lung in *N. brasiliensis* infection, Th2 cells maintained similar glycolytic gene expression, but lung Th2 cells appeared to have reduced glucose uptake. Given that cells from the lung are more potent cytokine producing cells, it appears unlikely that glycolysis is critical for Th2 effector function.

Despite this assertion, glycolysis may contribute to the development of pathogenic Th2 cells. Activated $CD4^+$ T cells from asthmatic patients, or malnourished mice, preferentially produce Th2 cytokines, and are associated with elevated glycolysis over activated cells from healthy controls[206,258]. Though I would argue glycolysis is not a key component of Th2 metabolism for cytokine production, it is possible

that glycolysis nonetheless promotes pathogenic cytokine production through indirect means. Increased glycolysis also correlated with stronger activation and a greater proliferative ability. Therefore, the pathogenicity of these Th2 cells may be caused by a combination of accelerated translation of cytokine mRNA and increased numbers of cytokine producing cells (Fig. 6.1).

Something not usually considered when studying T cell metabolism is the phenotype of naïve T cells. Metabolic activity is generally assessed in activated T cells, juxtaposed with different effector populations or similarly activated transgenic T cells. In Th2-mediated disease it may be that naïve $CD4^+$ T cells are predisposed to become more metabolic following stimulation. Metabolism in naïve T cells changes within minutes of activation[236]. One group queried how this was possible and discovered quiescent cells ignorant of antigen already possessed glycolytic mRNAs and ribosomal machinery poised for immediate protein synthesis after TCR ligation[310]. Therefore, in patients, characterization of unmanipulated, naïve $CD4^+$ T cells may be just as informative as studying activated populations. The route of disease may lie in the metabolic disposition of quiescent cells, and their propensity to activate or maintain elevated metabolism, and indirectly, cytokine production.

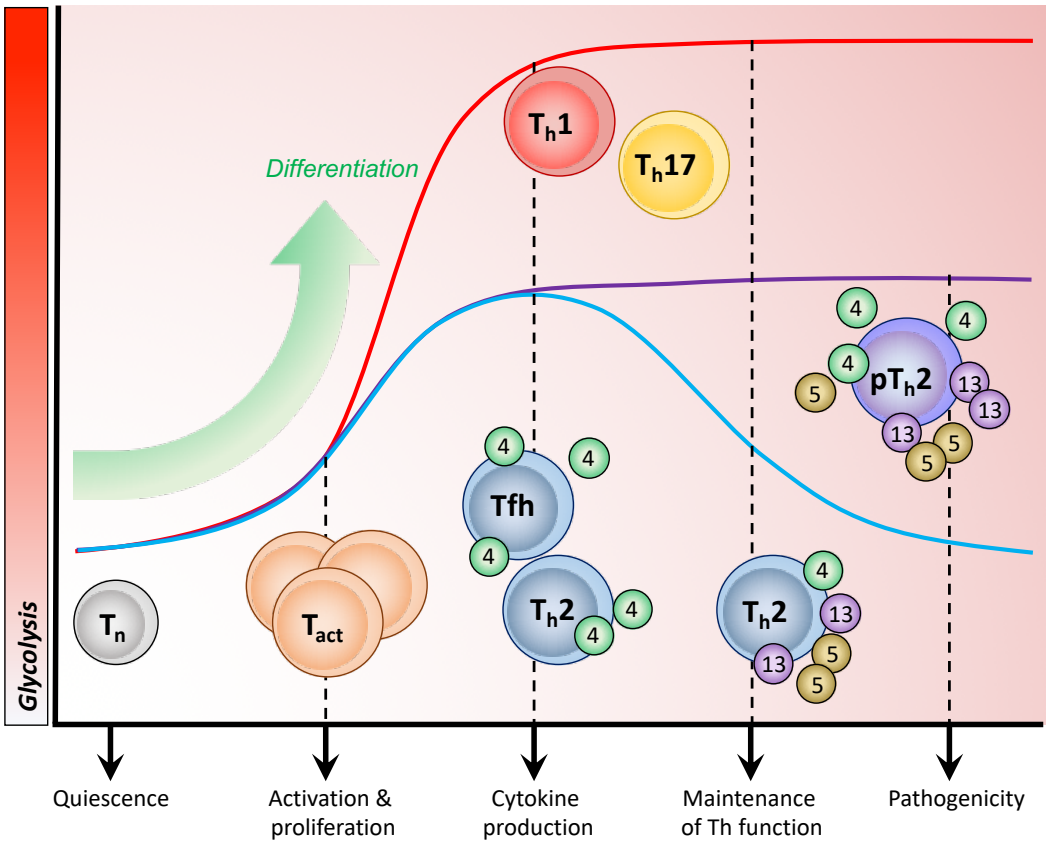

Figure 6.1 | Proposed model of glycolytic regulation of CD4$^+$ T cell function

Glycolysis is required for initial activation and polarization of Th2 cells, but continued Th2 cytokine expression appears independent of glycolysis and may require other pathways. In contrast, in Th1 and Th17 cells, glycolysis must be maintained for cytokine expression. Prolonged maintenance of high glycolysis in Th2 cells, however, could be a promoting factor of pathogenic (p)Th2 cells that are observed in atopic disease.

6.2 Metabolic regulation by helminths

Where glycolysis may contribute to pathogenic Th2 cell function, the converse may align with the ability of helminths to suppress Th2 responses during infection. In this context the Th2 response may be hindered by blocking the glycolytic checkpoint needed for activation and differentiation. Several hits in a proteomic analysis of molecules produced by *H. polygyrus* have a predicted metabolic function, including inhibiting host glycolysis[267]. The default pathway for $CD4^+$ T cell differentiation when glycolysis is blocked, or for mTOR deficient cells, is towards a Treg phenotype[156]. In line with this, infected individuals present with increased Treg numbers which correspond negatively to resistance[52]. Hence metabolic manipulation may be a mechanism in which helminths facilitate Treg induction. Indeed, Rick Maizels' group have identified and characterized a mimic of TGF-β produced by *H. polygyrus*, *Hp*-TGM[74,272]. Downstream signalling invoked by TGF-β coordinates metabolic changes that promote Treg differentiation[269,275]. In Chapter 4, I found that TGM similarly suppresses glycolysis during T cell activation (Fig. 4.7). However, when I examined the ability of total HES to alter Th2 metabolism, I did not consistently see an effect on glycolysis (Fig. 4.6). It could be that the potency of antibody-driven TCR stimulation *in vitro* overcomes the actions of any metabolically suppressive molecules in HES, which may be relatively dilute. Additionally, the products in HES are a result of *in vitro* cultivation and may differ in prevalence, or stability, *in vivo*.

However, directly assessing the impact of helminths on T cell metabolism *in vivo* is a difficult endeavour. To properly achieve this, more molecular characterization of helminth products would be needed to identify key metabolic regulators, which would require fractionation and large-scale synthesis. My approach instead was to evaluate metabolism in Th2 cells elicited in the absence of suppressive parasite-derived molecules produced by *H. polygyrus*, using house-dust mite and *N. brasiliensis* models. Th2 cells from these models would be anticipated to have higher glycolysis than observed for *H. polygyrus* infection, if *H. polygyrus* indeed produced molecules to target T cell metabolism. Gene expression of some glycolytic enzymes in Th2 cells isolated from HDM challenge or *N. brasiliensis* infection showed very minor, but significant, increases compared to naïve cells (Fig. 5.2 and 5.3). Though for the most part, gene expression appeared similar to that in *H. polygyrus* infection. The most notable difference in the acute models, compared to *H. polygyrus*, was the significantly increased expression of pyruvate kinase (*PKM2*), which could be meaningful, as it is the terminal enzyme in the pathway that produces pyruvate and ATP. Overall, the similarity between models suggests low glycolysis is an inherent metabolic feature of Th2 cells. Though, real-time metabolic measurements still need to be done to confirm the functional relevance of gene expression in *N. brasiliensis* or HDM activated Th2 cells before concluding this assertion.

It is also unknown how the molecules secreted and excreted by *H. polygyrus* change over its lifecycle in the host, or where they are active. The larvae begin

infection by burrowing into the submucosa, and at this point may release products that directly target neighbouring cells or may go systemic. Systemic spread from the larvae could be the case with TGM, as it is produced mainly during the larval stage, and an increase in $Foxp3^+$ cells is detected in the MLN during OVA challenge in mice infected with *H. polygyrus*[74]. Adult worms surface back into the lumen of the intestine where they remain attached by coiling around villi. During adulthood then, one might anticipate released products to target mainly epithelial cells, or cells of close proximity in the lamina propria. In the latter case, Th2 cells from the MLN might not experience the same level of suppression as in the small intestine. Previously, the technical challenges around isolating cells from the intestine during *H. polygyrus* infection made doing metabolic analysis of intestinal Th2 cells unfeasible. However, a protocol has been optimized in the lab for the successful digestion of helminth infected intestines[311], and it is of further interest to metabolically characterize intestinal Th2 cells.

Though glycolysis has been a central focus as a target by helminths in this study, the potential of helminths to manipulate other pathways must be taken into consideration. My results in Chapter 5 demonstrate that extracellular FA may have a prominent role in promoting tissue-Th2 cell function. This parallels many pieces of research that support a dichotomy between type 1 and type 2 responses for innate cells, in which glycolysis supports type 1 immunity while FAO supports type 2[120]. Interestingly, it has also been discovered that FA uptake and oxidation is critical to support helminth egg production[312]. Venom-allergen like (VAL) proteins

are lipid binding proteins that are also abundantly secreted by *H. polygyrus*[267,313]. The importance of VALs in infection is not yet understood, but they are highly conserved proteins among different helminth species[313]. It could be speculated that secretion of this family of lipid-binding proteins aids the acquisition of necessary fuel for helminth fecundity. This action could in parallel starve immune cells of critical fatty acids needed for effective parasite expulsion. VALs therefore may be an example of using metabolism to both restrict host immunity while simultaneously contributing to pathogen spread. Hence, the helminth molecules contributing to immuno-metabolic suppression are likely numerous and may work via diverse mechanisms.

6.3 Tissue control of Th2 cell FA metabolism

In Chapter 5 I found that Th2 cells selectively acquired FA, and moreover that this metabolic trait was most pronounced in $ST2^+$ cells, almost exclusively found in the lung (Fig. 5.4 and 5.5). From the existing immunometabolism literature I was able to hypothesize that PD-1 signalling alters Th2 metabolism to permit ST2 expression[83,125]. In support of this hypothesis, *ex vivo* stimulation with PD-L1 significantly increased the frequency and intensity of ST2 expression in Th2 cells (Fig. 5.11). However, this appeared to be at the expense of further proliferation, and, in the absence of IL-33 signalling, shut down IL-4 expression. Therefore, from these results I have proposed that PD-1 acts as a guardian in the tissue to prevent unnecessary Th2 responses, unless damage is present, causing the release of

alarmins. In the presence of these alarmins, Th2 cells can then produce cytokines to amplify the innate response, helminth expulsion, and repair (Fig. 6.2).

Further investigation is required to determine the exact contribution of PD-1 to metabolic regulation of Th2 cells. Ideally, this would be done using mice with a CD4-cre and floxed PD-1 gene (*Pdcd1*), which are commercially available. Using these mice, I would both assess their ability to express ST2 during infection, as well as their metabolic properties. I would predict ST2 expression to be abolished in Th2 cells, and therefore they would produce less cytokine in the tissue, and mice would become susceptible to long-term infection with helminths. Metabolically, if my model is correct, Th2 cells in these mice will fail to increase FA uptake and mitochondrial metabolism, and perhaps maintain elevated glycolysis. The necessity of PPARγ driven FA metabolism in inducing ST2 expression *in vivo* could then be tested using a variety of clinically used agonists to bypass the need for PD-1 signalling. Possibly a more therapeutically relevant, and readily available, approach would be to use a PD-1 blocking antibody.

The degree of impact that PD-1 and FA metabolism has on infection still needs to be established. A downfall in using *N. brasiliensis* infection as an experimental model is the rapid expulsion of the parasite, making it difficult to evaluate T cell dependent effects on worm burden. Therefore, *H. polygyrus* may be a better model as this parasite more successfully establishes infection. This would require some characterization of PD-1 and ST2 on $CD4^+$ T cells in the intestine beforehand. In

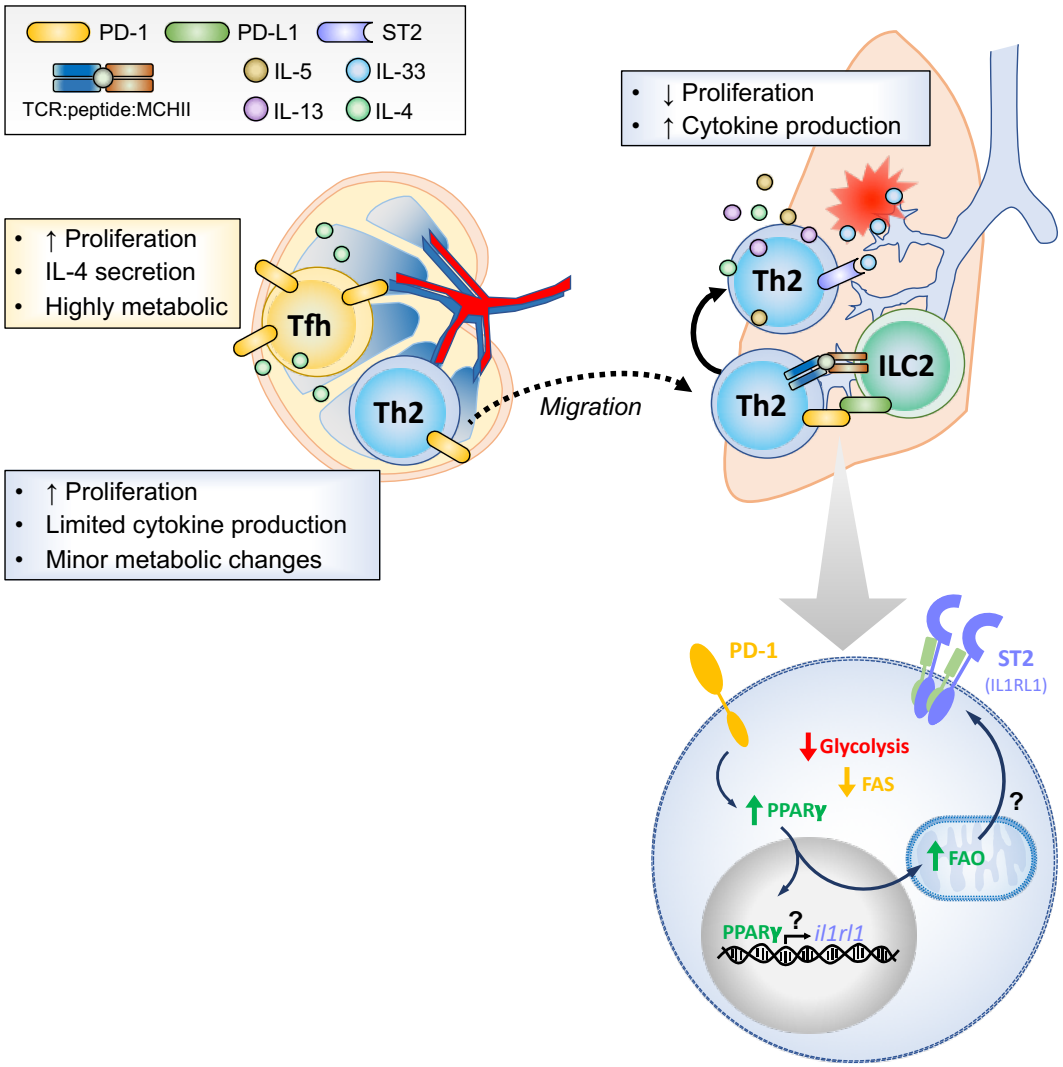

Figure 6.2 | Model for PD-1 control of tissue Th2 cell function

T cell activation leads to expression of PD-1 in Tfh and primed Th2 cells in the lymph node. Tfh cells become highly metabolic to survive a nutrient depleted environment, produce IL-4, and require PD-1 signalling to restrain aberrant expansion. Metabolic transitioning in primed Th2 cells supports activation, proliferation and migration to effector sites. Tissue-restricted PD-L1 signalling alongside secondary antigen recognition induces PPARγ expression, increases fatty acid oxidation and permits ST2 expression. PD-L1 simultaneously blocks proliferation to allow cytokine production without further expansion of effector Th2 cells.

regard to the role of FA metabolism in ST2 expression, experiments are underway to demonstrate that in the absence of external or cell-intrinsic free FA, PD-L1-induced ST2 expression is blocked. Lastly, it needs to be validated that PD-1 indeed alters metabolism in Th2 cells, which can be done through metabolite uptake and qPCR analysis of PD-L1 treated *ex vivo* cells, as well as with Seahorse analysis using αCD3 and PD-L1 coated plates to determine if FA are fuelling mitochondrial OXPHOS. Together, the aim of these experiments is to show that FAO directly contributes to ST2 expression, and that FAO is a consequence of PD-1 signalling, in Th2 cells isolated from helminth-infected mice.

6.3.1 Environmental control of T cell metabolism

Several recent reviews have proposed that the nutrient environment is a critical factor in regulating immune cell function, and the lung may represent a metabolic niche[314–317]. Therefore, whether the data I have presented here is in fact a universal trait of Th2 cells, or simply a consequence of the lung environment could be questioned. I plan to address this concern by determining if a similar, FA-dependent phenotype is adopted by Th1 cells during influenza A virus infection. Additionally, I aim to characterize Th2 metabolism in other tissues, namely the intestine.

A potentially unique property of the alveolar space is that glucose concentrations are maintained as low as 20-fold less than in the blood[318]. It has been proposed that pulmonary glucose transport is a defensive mechanism against opportunistic

bacterial infection and, as such, patients with hyperglycaemia, or forms of chronic lung disease, have elevated glucose concentrations in the airways that positively correlate with bacterial burdens in the respiratory tract[318–320]. Immunologically, macrophages dwelling in the alveolar space are unresponsive to IL-4 stimulation due to glycolytic restraints, which therefore could be a repercussion of low glucose availability in the airways[299]. A second unique aspect of the lung is the bulk supply of lipids available through surfactants[321]. Inadequate glucose and plentiful lipid availability could be a sensible rationale for Th2 to adopt a predominantly FA-fuelled metabolism. In support of this notion, Tregs in the visceral adipose tissue, presumably a rich source of lipids, also highly express PPARγ and ST2[322,323]. Environment may therefore be the major determinant of T cell metabolism at distinct anatomical sites.

While this argument may hold true for alveolar macrophages under homeostasis, migrating T cells should have ample glucose supply from the blood as they travel systemically to their destination. Additionally, in the case of *N. brasiliensis*, the site of infection undergoes severe damage, resulting in bleeding into the respiratory system for several days[324], which would increase local glucose concentrations. Low glycolysis is also maintained in primed Th2 cells from the lymph node prior to migration to the effector site suggesting glucose metabolism is independent of the tissue. Taken together, these data suggest that Th2 responses occur in the presence or absence of freely available glucose, and these concepts therefore support a model in which low glucose metabolism is an inherent trait of Th2 cells.

In contrast, I observed that Tfh cells from the draining LN during *N. brasiliensis* infection had significantly higher glucose uptake than naïve or Th2 cells. Tfh cells are thought to experience strong PD-1 signalling, and hence the opposite observation would be expected[223,302]. However, following immunization, germinal centres have been observed to have areas of hypoxia[309,325]. In situations of hypoxia, the low oxygen availability promotes glycolysis[177,309,326,327]. Therefore, low oxygen tension in the draining lymph node may override PD-1 signals that control T cell metabolism. Hypoxia could also explain the reduced mitochondrial fitness of *il4*-expressing cells in the MdLN (Fig. 5.8), due to the lack of oxygen to serve as terminal electron receptor. Furthermore, hypoxia and glycolysis promote progression through the cell-cycle[146,327], a potential rationale as to why PD-1 signalling is needed to restrain aberrant Tfh cell expansion. Epigenetically, increased glucose uptake may prevent opening of the *il1rl1* locus[308]. Hence, a number of ways in which the tissue environment can influence T cell metabolism and function have been illustrated.

The intestine is another prominent site where Th2 responses may occur. To my knowledge, ST2 expression has not been investigated on intestinal Th2 cells. However, the importance of ST2 in enteric type 2 responses is illustrated through the increased susceptibility of $ST2^{-/-}$ mice to intestinal helminths[73,282,284], and by IL-33 induced exacerbation of Th2-associated colitis[328,329]. As ILC2s express ST2 in the intestine, increased susceptibility to helminth infection in $ST2^{-/-}$ animals may

be partially due to reduced ILC2 activation. This is unlikely to be the full explanation, however, as ILC2s only provide partial protection in the absence of T cells[80]. Additionally, ST2$^+$ cells are found in the lung following primary intestinal *H. polygyrus* infection that provide protection to *N. brasiliensis* secondary infection[330]. It is unknown whether these cells expressed ST2 before or after lung entry, but it is possible that they originated from the intestine, the initial site of infection.

ST2 expression on Th2 cells therefore appears important for gut responses, but whether it is driven by PD-1, or whether Th2 cells acquire a similar metabolism as in the lung, still needs to be investigated. FAO is a characteristic feature of Tregs[143,176,180], which can also express ST2 in a PPARγ dependent manner[331,332]. ST2$^+$ Tregs have been found in the murine colon[288] and, although ST2-expressing Tregs are thought to be mostly thymically derived[289], colonic ST2$^+$ Tregs have heterogenous Helios expression suggesting that at least a portion acquire ST2 in the tissue[288]. Rapid FA uptake and oxidation is also characteristic of ILC2 residing in the small intestine lamina propria[285]. It has not been determined if ILC2 in the gut can express PD-L1 as they can in the lung, but it has been shown that they are required for optimal enteric Th2 responses to *H. polygyrus*[80]. Combined, these data convey that ST2 is critical in multiple tissues, and FA metabolism is shared amongst different ST2$^+$ immune populations in the intestine, suggesting PD-1 driven ST2 expression is also plausible in intestinal Th2 cells. As the small intestine is a major site of lipid absorption, I would predict it to be a suitable

environment for optimal Th2 induction, if indeed FA metabolism is a widespread requirement for Th2 function.

6.3.2 Whole body metabolism, infection and immunity

Ultimately, like any activated T cell, a Th2 cell is stimulated through the TCR to proliferate and produce cytokine. What reason would Th2 cells have for differing in their metabolic requirements? Organismal, or whole-body, metabolism is dramatically altered during infection, and it has been hypothesized that this occurs to optimize the immune response (Fig. 6.3). Viral infection and bacterial sepsis both induce patient hyperglycaemia providing ample fuel for inflammatory macrophage and Th1 glycolysis[333,334]. Helminths on the other hand modulate host metabolism to improve insulin sensitivity, lowering blood glucose, which would make it more challenging for a glycolysis-dependent T cell to perform its duty[248,249,333,335,336]. Furthermore, helminths lower serum cholesterol and protect against obesity when mice are fed a high fat diet[337–339]. Host metabolism also has an impact on the outcome of helminth infection, as obesity prone mice are less resistant to infection than lean mice, arguing there is a direct link between whole-body metabolism and Th2 responses[340].

One conceptual explanation for this link could be that the type 2 immune response engages in a metabolic competition in an attempt of starve the parasites. There indeed is some indication that helminths are highly dependent on FA, particularly for egg production[312]. VAL proteins are some of the most highly conserved and

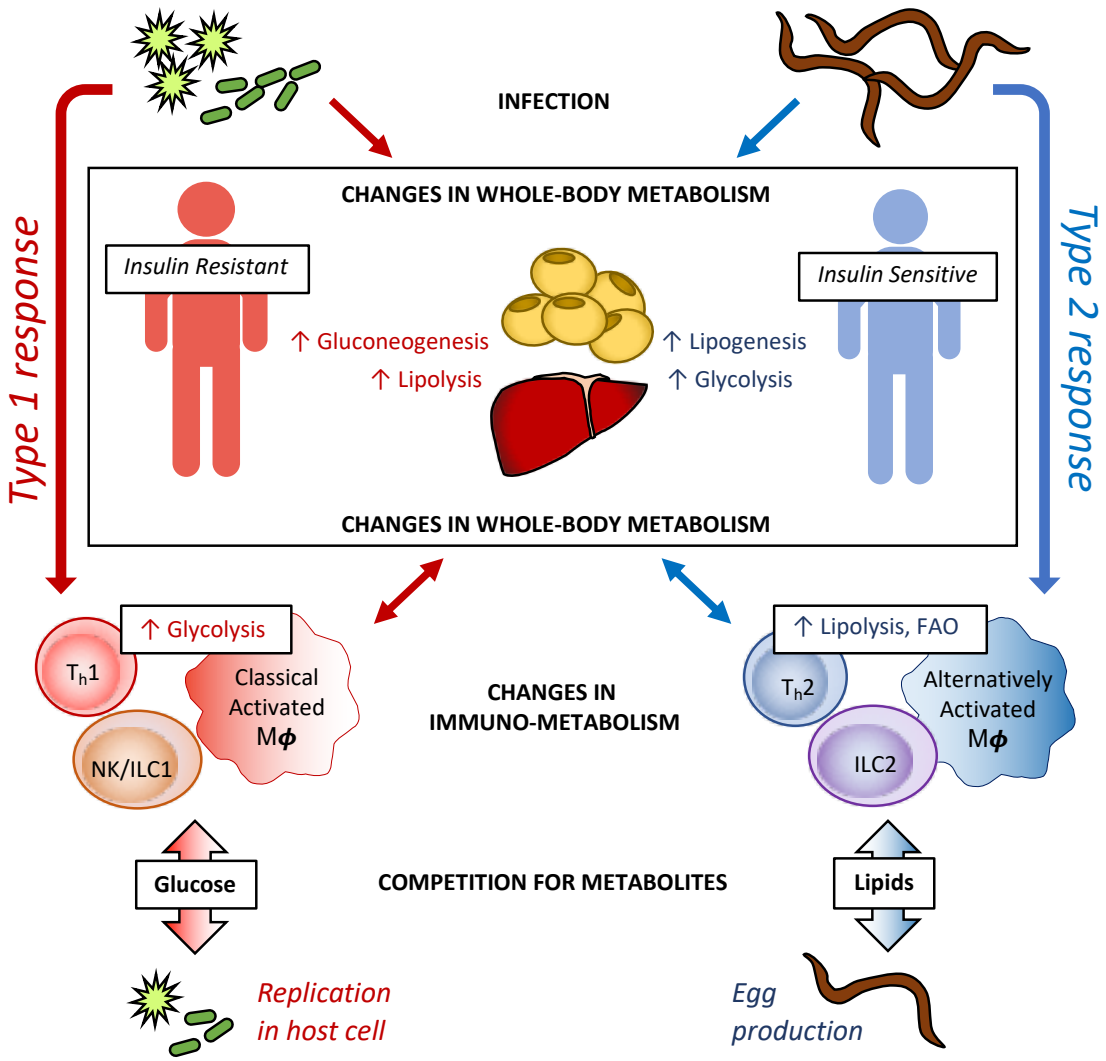

Figure 6.3 | whole-body metabolism, infection and metabolic competition

Whole body metabolism is affected differently by distinct classes of pathogens. Viral and bacterial infections are associated with insulin resistance, and therefore increased circulating glucose concentrations. Helminths cause hosts to become sensitive to insulin, which lowers blood-glucose levels. These changes in host metabolism may be reflective of the needs of the immune system to adequately control infection or, conversely, may be a beneficial consequence for pathogen survival and replication. Adapted from Chawla, Nguyen and Goh[333].

secreted proteins across helminth species, and though their role in infection is unknown, they contain multiple fatty acid binding sites[267,313]. However, rather than the immune response restricting available nutrients used by the parasites, it could also be argued that through co-evolution helminths have developed means to alter host metabolism to their own competitive advantage. Tangentially, in the absence of helminth infection, does the wider link between organismal metabolism and the immune response suggest the western diet, one that is rich in fats, is contributing to increases in atopic disease by providing excess fuel to support type 2 immunity?

6.5 Concluding statements

In summary, I have characterized a novel metabolic phenotype for Th2 cells *in vivo*. In contrast to the current literature, my data show that Th2 cells use minimal glycolysis. Instead, Th2 cells dynamically regulate FA metabolism. By comparing metabolism in Th2 cells from the MdLN and lung, I showed that lung Th2 cells maintain low glucose metabolism but sharply increase FA uptake and mitochondrial metabolism. I have hypothesized that PD-1 controls a metabolic switch in Th2 cells in the lung and promotes Th2 function by upregulating the IL-33 receptor, ST2. This is supported by the strong co-expression of both receptors, and the correlation between FA metabolism and PD-1^{+}ST2^{+} cells. Furthermore, direct stimulation of PD-1 led to increased ST2 expression in Th2 cells. Preliminary data also suggest that helminths may target host metabolism as means of immune evasion, but more work is needed to determine the pathways targeted, and

molecules responsible. The current standard for understanding T cell metabolism is to use *in vitro* activated cultures. My data serves as a cautionary tale in interpreting *in vitro* results and predicting their translation *in vivo*. My work also contributes important insight into the workings of Th2 biology, that could be taken into consideration when designing future therapies against helminth infection or atopic disease.

Bibliography

1. Zhu, J., Yamane, H. & Paul, W. E. Differentiation of effector CD4 T cell populations (*). *Annu. Rev. Immunol.* **28**, 445–89 (2010).

2. Almeida, L., Lochner, M., Berod, L. & Sparwasser, T. Metabolic pathways in T cell activation and lineage differentiation. *Semin. Immunol.* **28**, 514–524 (2016).

3. Shah, D. K. & Zúñiga-Pflücker, J. C. An overview of the intrathymic intricacies of T cell development. *J. Immunol. Baltim. Md 1950* **192**, 4017–4023 (2014).

4. Klein, L., Hinterberger, M., Wirnsberger, G. & Kyewski, B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat. Rev. Immunol.* **9**, 833–844 (2009).

5. Koch, U. & Radtke, F. Mechanisms of T Cell Development and Transformation. *Annu. Rev. Cell Dev. Biol.* **27**, 539–562 (2011).

6. Girard, J.-P., Moussion, C. & Förster, R. HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat. Rev. Immunol.* **12**, 762–773 (2012).

7. Mueller, S. N. & Germain, R. N. Stromal cell contributions to the homeostasis and functionality of the immune system. *Nat. Rev. Immunol.* **9**, 618–629 (2009).

8. Cyster, J. G. & Allen, C. D. C. B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell* **177**, 524–540 (2019).

9. Itano, A. A. & Jenkins, M. K. Antigen presentation to naive CD4 T cells in the lymph node. *Nat. Immunol.* **4**, 733 (2003).

10. Gentek, R. & Bajénoff, M. Lymph Node Stroma Dynamics and Approaches for Their Visualization. *Trends Immunol.* **38**, 236–247 (2017).

11. Smith-Garvin, J. E., Koretzky, G. A. & Jordan, M. S. T Cell Activation. *Annu. Rev. Immunol.* **27**, 591–619 (2009).

12. Esensten, J. H., Helou, Y. A., Chopra, G., Weiss, A. & Bluestone, J. A. CD28 costimulation: from mechanism to therapy. *Immunity* **44**, 973–988 (2016).

13. Hirahara, K. *et al.* Mechanisms underlying helper T-cell plasticity: implications for immune-mediated disease. *J. Allergy Clin. Immunol.* **131**, 1276–1287 (2013).

14. Luckheeram, R. V., Zhou, R., Verma, A. D. & Xia, B. CD4+T Cells: Differentiation and Functions. *Clin. Dev. Immunol.* **2012**, (2012).

15. Patel, D. D. & Kuchroo, V. K. Th17 Cell Pathway in Human Immunity: Lessons from Genetics and Therapeutic Interventions. *Immunity* **43**, 1040–1051 (2015).

16. Stritesky, G. L., Yeh, N. & Kaplan, M. H. IL-23 promotes maintenance but not commitment to the Th17 lineage. *J. Immunol. Baltim. Md 1950* **181**, 5948–5955 (2008).

17. Lee, Y. *et al.* Induction and molecular signature of pathogenic TH17 cells. *Nat. Immunol.* **13**, 991–999 (2012).

18. Walker, J. A. & McKenzie, A. N. J. TH2 cell development and function. *Nat. Rev. Immunol.* nri.2017.118-nri.2017.118 (2017). doi:10.1038/nri.2017.118

19. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol. Baltim. Md 1950* **136**, 2348–57 (1986).

20. van Panhuys, N. *et al.* In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 12423–12428 (2008).

21. Lawrence, R. A., Gray, C. A., Osborne, J. & Maizels, R. M. Nippostrongylus brasiliensis: cytokine responses and nematode expulsion in normal and IL-4-deficient mice. *Exp. Parasitol.* **84**, 65–73 (1996).

22. Phythian-Adams, A. T. *et al.* CD11c depletion severely disrupts Th2 induction and development in vivo. *J. Exp. Med.* **207**, 2089–2096 (2010).

23. Connor, L. M., Tang, S.-C., Camberis, M., Le Gros, G. & Ronchese, F. Helminth-conditioned dendritic cells prime CD4+ T cells to IL-4 production in vivo. *J. Immunol. Baltim. Md 1950* **193**, 2709–2717 (2014).

24. Ito, T. *et al.* TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* **202**, 1213–1223 (2005).

25. Okamoto, M. *et al.* Jagged1 on dendritic cells and Notch on CD4+ T cells initiate lung allergic responsiveness by inducing IL-4 production. *J. Immunol. Baltim. Md 1950* **183**, 2995–3003 (2009).

26. Jenkins, S. J., Perona-Wright, G., Worsley, A. G. F., Ishii, N. & MacDonald, A. S. Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction in vivo. *J. Immunol. Baltim. Md 1950* **179**, 3515–3523 (2007).

27. Tindemans, I. *et al.* Notch signaling in T cells is essential for allergic airway inflammation, but expression of the Notch ligands Jagged 1 and Jagged 2 on dendritic cells is dispensable. *J. Allergy Clin. Immunol.* **140**, 1079–1089 (2017).

28. Crotty, S. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* **29**, 621–663 (2011).

29. Reinhardt, R. L., Liang, H.-E. & Locksley, R. M. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* **10**, 385–393 (2009).

30. King, I. L. & Mohrs, M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J. Exp. Med.* **206**, 1001–7 (2009).

31. Ballesteros-Tato, A. *et al.* T Follicular Helper Cell Plasticity Shapes Pathogenic T Helper 2 Cell-Mediated Immunity to Inhaled House Dust Mite. *Immunity* **44**, 259–273 (2016).

32. Zaretsky, A. G. *et al.* T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J. Exp. Med.* **206**, (2009).

33. Nakayamada, S. *et al.* Early Th1 Cell Differentiation Is Marked by A Tfh-like Cell Transition. *Immunity* **35**, 919–931 (2011).

34. Nurieva, R. I. *et al.* Bcl6 Mediates the Development of T Follicular Helper Cells. *Science* **325**, 1001–1005 (2009).

35. Yu, D. *et al.* The Transcriptional Repressor Bcl-6 Directs T Follicular Helper Cell Lineage Commitment. *Immunity* **31**, 457–468 (2009).

36. Plitas, G. & Rudensky, A. Y. Regulatory T Cells: Differentiation and Function. *Cancer Immunol. Res.* **4**, 721–725 (2016).

37. Schmidt, A., Oberle, N. & Krammer, P. H. Molecular mechanisms of treg-mediated T cell suppression. *Front. Immunol.* **3**, 51–51 (2012).

38. Duhen, T., Duhen, R., Lanzavecchia, A., Sallusto, F. & Campbell, D. J. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. *Blood* **119**, 4430–4440 (2012).

39. Ley, K. The second touch hypothesis: T cell activation, homing and polarization. *F1000Research* **3**, (2014).

40. Krummel, M. F., Bartumeus, F. & Gérard, A. T cell migration, search strategies and mechanisms. *Nat. Rev. Immunol.* **16**, 193–201 (2016).

41. Mohrs, K., Wakil, A. E., Killeen, N., Locksley, R. M. & Mohrs, M. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity* **23**, 419–29 (2005).

42. Minutti, C. M. *et al.* Epidermal Growth Factor Receptor Expression Licenses Type-2 Helper T Cells to Function in a T Cell Receptor-Independent Fashion. *Immunity* **47**, 710-722.e6 (2017).

43. Van Dyken, S. J. *et al.* A tissue checkpoint regulates type 2 immunity. *Nat. Immunol.* **17**, 1381–1387 (2016).

44. McKinstry, K. K., Strutt, T. M. & Swain, S. L. Regulation of CD4+ T-cell contraction during pathogen challenge. *Immunol. Rev.* **236**, 110–124 (2010).

45. Gasper, D. J., Tejera, M. M. & Suresh, M. CD4 T-Cell Memory Generation and Maintenance. *Crit. Rev. Immunol.* **34**, 121–146 (2014).

46. Pearce, E. L. & Pearce, E. J. Metabolic pathways in immune cell activation and quiescence. *Immunity* **38**, 633–43 (2013).

47. Wherry, E. J. T cell exhaustion. *Nat. Immunol.* **12**, 492–499 (2011).

48. Okada, H., Kuhn, C., Feillet, H. & Bach, J.-F. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clin. Exp. Immunol.* **160**, 1–9 (2010).

49. Hotez, P. J. *et al.* Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118**, 1311–21 (2008).

50. Charlier, J., van der Voort, M., Kenyon, F., Skuce, P. & Vercruysse, J. Chasing helminths and their economic impact on farmed ruminants. *Trends Parasitol.* **30**, 361–367 (2014).

51. Salgame, P., Yap, G. S. & Gause, W. C. Effect of helminth-induced immunity on infections with microbial pathogens. *Nat. Immunol.* **14**, 1118–26 (2013).

52. McSorley, H. J. & Maizels, R. M. Helminth infections and host immune regulation. *Clin. Microbiol. Rev.* **25**, 585–608 (2012).

53. Urban, J. F., Katona, I. M., Paul, W. E. & Finkelman, F. D. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proc. Natl. Acad. Sci. U. S. A.* **88**, 5513–7 (1991).

54. Urban, J. F. *et al.* IL-13, IL-4Rα, and Stat6 Are Required for the Expulsion of the Gastrointestinal Nematode Parasite Nippostrongylus brasiliensis. *Immunity* **8**, 255–264 (1998).

55. Lanusse, C. *et al.* Strategies to Optimize the Efficacy of Anthelmintic Drugs in Ruminants. *Trends Parasitol.* **34**, 664–682 (2018).

56. Hewitson, J. P. & Maizels, R. M. Vaccination against helminth parasite infections. *Expert Rev. Vaccines* **13**, 473–487 (2014).

57. Pawankar, R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ. J.* **7**, 55 (2014).

58. Thomsen, S. F. Epidemiology and natural history of atopic diseases. *Eur. Clin. Respir. J.* **2**, (2015).

59. Galli, S. J., Tsai, M. & Piliponsky, A. M. The development of allergic inflammation. *Nature* **454**, 445–454 (2008).

60. Caminati, M., Pham, D. L., Bagnasco, D. & Canonica, G. W. Type 2 immunity in asthma. *World Allergy Organ. J.* **11**, 13 (2018).

61. Endo, Y., Hirahara, K., Yagi, R., Tumes, D. J. & Nakayama, T. Pathogenic memory type Th2 cells in allergic inflammation. *Trends Immunol.* **35**, 69–78 (2014).

62. Gregory, L. G. & Lloyd, C. M. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol.* **32**, 402–411 (2011).

63. Correale, J. & Farez, M. Association between parasite infection and immune responses in multiple sclerosis. *Ann. Neurol.* **61**, 97–108 (2007).

64. Correale, J. & Farez, M. F. The impact of parasite infections on the course of multiple sclerosis. *J. Neuroimmunol.* **233**, 6–11 (2011).

65. Fleming, J. O. & Weinstock, J. V. Clinical trials of helminth therapy in autoimmune diseases: rationale and findings. *Parasite Immunol.* **37**, 277–92 (2015).

66. Briggs, N., Weatherhead, J., Sastry, K. J. & Hotez, P. J. The Hygiene Hypothesis and Its Inconvenient Truths about Helminth Infections. *PLoS Negl. Trop. Dis.* **10**, (2016).

67. Maizels, R. M., Smits, H. H. & McSorley, H. J. Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. *Immunity* **49**, 801–818 (2018).

68. Reynolds, L. A., Filbey, K. J. & Maizels, R. M. Immunity to the model intestinal helminth parasite Heligmosomoides polygyrus. *Semin. Immunopathol.* **34**, 829–46 (2012).

69. Camberis, M., Gros, G. L. & Urban, J. Animal Model of Nippostrongylus brasiliensis and Heligmosomoides polygyrus. *Curr. Protoc. Immunol.* **55**, 19.12.1-19.12.27 (2003).

70. Finney, C. A. M., Taylor, M. D., Wilson, M. S. & Maizels, R. M. Expansion and activation of CD4+CD25+ regulatory T cells in Heligmosomoides polygyrus infection. *Eur. J. Immunol.* **37**, 1874–1886 (2007).

71. Smith, K. A. *et al.* Low-level regulatory T-cell activity is essential for functional type-2 effector immunity to expel gastrointestinal helminths. *Mucosal Immunol.* **9**, 428–443 (2015).

72. Osbourn, M. *et al.* HpARI Protein Secreted by a Helminth Parasite Suppresses Interleukin-33. *Immunity* **47**, 739-751.e5 (2017).

73. Coakley, G. *et al.* Extracellular Vesicles from a Helminth Parasite Suppress Macrophage Activation and Constitute an Effective Vaccine for Protective Immunity. *Cell Rep.* **19**, 1545–1557 (2017).

74. Grainger, J. R. *et al.* Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. *J. Exp. Med.* **207**, 2331–41 (2010).

75. Harvie, M., Camberis, M. & Le Gros, G. Development of CD4 T Cell Dependent Immunity Against N. brasiliensis Infection. *Front. Immunol.* **4**, 74–74 (2013).

76. Sorobetea, D., Svensson-Frej, M. & Grencis, R. Immunity to gastrointestinal nematode infections. *Mucosal Immunol.* **11**, 304–315 (2018).

77. Nadjsombati, M. S. *et al.* Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. *Immunity* **49**, 33-41.e7 (2018).

78. Martin, N. T. & Martin, M. U. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat. Immunol.* **17**, 122–131 (2016).

79. Harris, N. L. & Loke, P. Recent Advances in Type-2-Cell-Mediated Immunity: Insights from Helminth Infection. *Immunity* **47**, 1024–1036 (2017).

80. Pelly, V. S. *et al.* IL-4-producing ILC2s are required for the differentiation of T_H2 cells following *Heligmosomoides polygyrus* infection. *Mucosal Immunol.* **9**, 1407–1417 (2016).

81. Neill, D. R. *et al.* Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* **464**, 1367–1370 (2010).

82. Oliphant, C. J. *et al.* MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity* **41**, 283–295 (2014).

83. Schwartz, C. *et al.* ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control. *J. Exp. Med.* **214**, 2507–2521 (2017).

84. Schlitzer, A. *et al.* IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses. *Immunity* **38**, 970–983 (2013).

85. Williams, J. W. *et al.* Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat. Commun.* **4**, 2990 (2013).

86. Everts, B. *et al.* Migratory CD103+ dendritic cells suppress helminth-driven type 2 immunity through constitutive expression of IL-12. *J. Exp. Med.* **213**, 35–51 (2016).

87. Mayer, J. U. *et al.* Different populations of $CD11b^+$ dendritic cells drive Th2 responses in the small intestine and colon. *Nat. Commun.* **8**, 15820 (2017).

88. Hussaarts, L., Yazdanbakhsh, M. & Guigas, B. Priming Dendritic Cells for Th2 Polarization: Lessons Learned from Helminths and Implications for Metabolic Disorders. *Front. Immunol.* **5**, (2014).

89. Reynolds, L. A. *et al.* MyD88 Signaling Inhibits Protective Immunity to the Gastrointestinal Helminth Parasite Heligmosomoides polygyrus. *J. Immunol. Author Choice* **193**, 2984–2993 (2014).

90. Everts, B. *et al.* Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *J. Exp. Med.* **206**, 1673–1680 (2009).

91. Massacand, J. C. *et al.* Helminth products bypass the need for TSLP in Th2 immune responses by directly modulating dendritic cell function. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 13968–73 (2009).

92. Rank, M. A. *et al.* IL-33-activated dendritic cells induce an atypical TH2-type response. *J. Allergy Clin. Immunol.* **123**, 1047–1054 (2009).

93. Matta, B. M. *et al.* IL-33 is an unconventional Alarmin that stimulates IL-2 secretion by dendritic cells to selectively expand IL-33R/ST2+ regulatory T cells. *J. Immunol. Baltim. Md 1950* **193**, 4010–4020 (2014).

94. Halim, T. Y. F. *et al.* Group 2 Innate Lymphoid Cells Are Critical for the Initiation of Adaptive T Helper 2 Cell-Mediated Allergic Lung Inflammation. *Immunity* **40**, 425–435 (2014).

95. León, B. *et al.* Regulation of TH2 development by CXCR5+ dendritic cells and lymphotoxin-expressing B cells. *Nat. Immunol.* **13**, 681–690 (2012).

96. Liu, Z. *et al.* Nippostrongylus brasiliensis Can Induce B7-Independent Antigen-Specific Development of IL-4-Producing T Cells from Naive CD4 T Cells In Vivo. *J. Immunol.* **169**, (2002).

97. Gause, W. C. *et al.* CD28 dependence of T cell differentiation to IL-4 production varies with the particular type 2 immune response. *J. Immunol. Baltim. Md 1950* **158**, 4082–4087 (1997).

98. Rudulier, C. D., McKinstry, K. K., Al-Yassin, G. A., Kroeger, D. R. & Bretscher, P. A. The Number of Responding CD4 T Cells and the Dose of Antigen Conjointly Determine the Th1/Th2 Phenotype by Modulating B7/CD28 Interactions. *J. Immunol.* **192**, 5140–5150 (2014).

99. Milner, J. D., Fazilleau, N., McHeyzer-Williams, M. & Paul, W. Cutting edge: lack of high affinity competition for peptide in polyclonal CD4+ responses unmasks IL-4 production. *J. Immunol. Baltim. Md 1950* **184**, 6569–73 (2010).

100. Rogers, P. R. & Croft, M. CD28, Ox-40, LFA-1, and CD4 modulation of Th1/Th2 differentiation is directly dependent on the dose of antigen. *J. Immunol. Baltim. Md 1950* **164**, 2955–2963 (2000).

101. Aki, D. *et al.* The E3 ligases Itch and WWP2 cooperate to limit TH2 differentiation by enhancing signaling through the TCR. *Nat. Immunol.* **19**, 766–775 (2018).

102. Schuijs, M. J., Hammad, H. & Lambrecht, B. N. Professional and 'Amateur' Antigen-Presenting Cells In Type 2 Immunity. *Trends Immunol.* **40**, 22–34 (2019).

103. Halim, T. Y. F. *et al.* Tissue-Restricted Adaptive Type 2 Immunity Is Orchestrated by Expression of the Costimulatory Molecule OX40L on Group 2 Innate Lymphoid Cells. *Immunity* **48**, 1195-1207.e6 (2018).

104. Hepworth, M. R. *et al.* Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4+ T cells. *Science* **348**, 1031–1035 (2015).

105. Cliffe, L. J. *et al.* Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science* **308**, 1463–1465 (2005).

106. Hasnain, S. Z. *et al.* Mucin gene deficiency in mice impairs host resistance to an enteric parasitic infection. *Gastroenterology* **138**, 1763–1771 (2010).

107. Hasnain, S. Z. *et al.* Muc5ac: a critical component mediating the rejection of enteric nematodes. *J. Exp. Med.* **208**, 893–900 (2011).

108. McDermott, J. R. *et al.* Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 7761–7766 (2003).

109. Schmidt, S. *et al.* Nippostrongylus-induced intestinal hypercontractility requires IL-4 receptor alpha-responsiveness by T cells in mice. *PloS One* **7**, e52211 (2012).

110. Allen, J. E. & Sutherland, T. E. Host protective roles of type 2 immunity: parasite killing and tissue repair, flip sides of the same coin. *Semin. Immunol.* **26**, 329–40 (2014).

111. Knott, M. L. *et al.* Impaired resistance in early secondary Nippostrongylus brasiliensis infections in mice with defective eosinophilopoeisis. *Int. J. Parasitol.* **37**, 1367–1378 (2007).

112. Dent, L. A. *et al.* Interleukin-5 transgenic mice show enhanced resistance to primary infections with Nippostrongylus brasiliensis but not primary infections with Toxocara canis. *Infect. Immun.* **67**, 989–993 (1999).

113. Batugedara, H. M. *et al.* Hematopoietic cell-derived RELMα regulates hookworm immunity through effects on macrophages. *J. Leukoc. Biol.* **104**, 855–869 (2018).

114. Krljanac, B. *et al.* RELMα-expressing macrophages protect against fatal lung damage and reduce parasite burden during helminth infection. *Sci. Immunol.* **4**, (2019).

115. Knipper, J. A. *et al.* Interleukin-4 Receptor α Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair. *Immunity* **43**, 803–816 (2015).

116. Anthony, R. M. *et al.* Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nat. Med.* **12**, 955–60 (2006).

117. Obata-Ninomiya, K. *et al.* CXCR6+ST2+ memory T helper 2 cells induced the expression of major basic protein in eosinophils to reduce the fecundity of helminth. *Proc. Natl. Acad. Sci.* **115**, E9849–E9858 (2018).

118. Maizels, R. M., Hewitson, J. P. & Smith, K. A. Susceptibility and immunity to helminth parasites. *Curr. Opin. Immunol.* **24**, 459–466 (2012).

119. Bieren, J. E. *et al.* Antibodies Trap Tissue Migrating Helminth Larvae and Prevent Tissue Damage by Driving IL-4Rα-Independent Alternative Differentiation of Macrophages. *PLOS Pathog.* **9**, e1003771 (2013).

120. O'Neill, L. A. J. & Pearce, E. J. Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* **213**, 15–23 (2015).

121. Geltink, R. I. K., Kyle, R. L. & Pearce, E. L. Unraveling the Complex Interplay Between T Cell Metabolism and Function. *Annu. Rev. Immunol.* **36**, 461–488 (2018).

122. O'Brien, K. L. & Finlay, D. K. Immunometabolism and natural killer cell responses. *Nat. Rev. Immunol.* **19**, 282–290 (2019).

123. Li, J., Kim, S. G. & Blenis, J. Rapamycin: one drug, many effects. *Cell Metab.* **19**, 373–379 (2014).

124. Kornberg, M. D. *et al.* Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science* **360**, 449–453 (2018).

125. Patsoukis, N. *et al.* PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat. Commun.* **6**, 6692–6692 (2015).

126. O'Neill, L. A. J., Kishton, R. J. & Rathmell, J. A guide to immunometabolism for immunologists. *Nat. Rev. Immunol.* **16**, 553–565 (2016).

127. Macintyre, A. N. *et al.* The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab.* **20**, 61–72 (2014).

128. Puleston, D. J., Villa, M. & Pearce, E. L. Ancillary Activity: Beyond Core Metabolism in Immune Cells. *Cell Metab.* **26**, 131–141 (2017).

129. Williams, N. C. & O'Neill, L. A. J. A Role for the Krebs Cycle Intermediate Citrate in Metabolic Reprogramming in Innate Immunity and Inflammation. *Front. Immunol.* **9**, (2018).

130. Abdelmagid, S. A. *et al.* Comprehensive Profiling of Plasma Fatty Acid Concentrations in Young Healthy Canadian Adults. *PLoS ONE* **10**, (2015).

131. Glatz, J. F. C., Luiken, J. J. F. P. & Bonen, A. Membrane Fatty Acid Transporters as Regulators of Lipid Metabolism: Implications for Metabolic Disease. *Physiol. Rev.* **90**, 367–417 (2010).

132. Huang, S. C.-C. *et al.* Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat. Immunol.* **15**, 846–55 (2014).

133. Hotamisligil, G. S. & Bernlohr, D. A. Metabolic functions of FABPs— mechanisms and therapeutic implications. *Nat. Rev. Endocrinol.* **11**, 592–605 (2015).

134. Yang, L., Venneti, S. & Nagrath, D. Glutaminolysis: A Hallmark of Cancer Metabolism. *Annu. Rev. Biomed. Eng.* **19**, 163–194 (2017).

135. Nakaya, M. *et al.* Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* **40**, 692–705 (2014).

136. Carr, E. L. *et al.* Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. *J. Immunol. Baltim. Md 1950* **185**, 1037–44 (2010).

137. Blagih, J. *et al.* The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* **42**, 41–54 (2015).

138. Buck, M. D., O'Sullivan, D. & Pearce, E. L. T cell metabolism drives immunity. *J. Exp. Med.* jem.20151159--jem.20151159- (2015). doi:10.1084/jem.20151159

139. Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029–33 (2009).

140. Wang, R. *et al.* The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–82 (2011).

141. Jacobs, S. R. *et al.* Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *J. Immunol.* **180**, 4476–4486 (2008).

142. Frauwirth, K. A. *et al.* The CD28 signaling pathway regulates glucose metabolism. *Immunity* **16**, 769–77 (2002).

143. Berod, L. *et al.* De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat. Med.* **20**, 1327–33 (2014).

144. O'Sullivan, D. *et al.* Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* **41**, 75–88 (2014).

145. Klein Geltink, R. I. *et al.* Mitochondrial Priming by CD28. *Cell* **0**, (2017).

146. Chang, C.-H. *et al.* Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* **153**, 1239–1251 (2013).

147. Angela, M. *et al.* Fatty acid metabolic reprogramming via mTOR-mediated inductions of PPARγ directs early activation of T cells. *Nat. Commun.* **7**, 13683–13683 (2016).

148. Buck, M. D. *et al.* Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. *Cell* **166**, 63–76 (2016).

149. Ron-Harel, N. *et al.* Mitochondrial Biogenesis and Proteome Remodeling Promote One-Carbon Metabolism for T Cell Activation. *Cell Metab.* **24**, 104–117 (2016).

150. Sena, L. A. *et al.* Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **38**, 225–36 (2013).

151. Bengsch, B. *et al.* Bioenergetic Insufficiencies Due to Metabolic Alterations Regulated by the Inhibitory Receptor PD-1 Are an Early Driver of CD8+ T Cell Exhaustion. *Immunity* 358–73 (2015).

152. Scharping, N. E. *et al.* The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. *Immunity* **45**, 374–388 (2016).

153. Bettonville, M. *et al.* Long-term antigen exposure irreversibly modifies metabolic requirements for T cell function. *eLife* **7**, e30938 (2018).

154. Pollizzi, K. N. & Powell, J. D. Regulation of T cells by mTOR: the known knowns and the known unknowns. *Trends Immunol.* **36**, 13–20 (2015).

155. Saxton, R. A. & Sabatini, D. M. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **168**, 960–976 (2017).

156. Delgoffe, G. M. *et al.* The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* **30**, 832–44 (2009).

157. Colombetti, S., Basso, V., Mueller, D. L. & Mondino, A. Prolonged TCR/CD28 engagement drives IL-2-independent T cell clonal expansion through signaling mediated by the mammalian target of rapamycin. *J. Immunol. Baltim. Md 1950* **176**, 2730–2738 (2006).

158. Düvel, K. *et al.* Activation of a Metabolic Gene Regulatory Network Downstream of mTOR Complex 1. *Mol. Cell* **39**, 171–183 (2010).

159. Kim, J., Tchernyshyov, I., Semenza, G. L. & Dang, C. V. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* **3**, 177–185 (2006).

160. Jacinto, E. *et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* **6**, 1122–1128 (2004).

161. Kishore, M. *et al.* Regulatory T Cell Migration Is Dependent on Glucokinase-Mediated Glycolysis. *Immunity* **47**, 875-889.e10 (2017).

162. Kim, J., Kundu, M., Viollet, B. & Guan, K.-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132–141 (2011).

163. Kidani, Y. *et al.* Sterol regulatory element–binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat. Immunol.* **14**, 489–499 (2013).

164. Chawla, A. Control of macrophage activation and function by PPARs. *Circ. Res.* **106**, 1559–69 (2010).

165. Varga, T., Czimmerer, Z. & Nagy, L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim. Biophys. Acta* **1812**, 1007–1022 (2011).

166. Odegaard, J. I. *et al.* Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. *Nature* **447**, 1116–1120 (2007).

167. Liang, H. & Ward, W. F. PGC-1α: a key regulator of energy metabolism. *Adv. Physiol. Educ.* **30**, 145–151 (2006).

168. Choi, J.-M. & Bothwell, A. L. M. The Nuclear Receptor PPARs as Important Regulators of T-Cell Functions and Autoimmune Diseases. *Mol. Cells* **33**, 217–222 (2012).

169. Clark, R. B. *et al.* The Nuclear Receptor PPARγ and Immunoregulation: PPARγ Mediates Inhibition of Helper T Cell Responses. *J. Immunol.* **164**, 1364–1371 (2000).

170. Guri, A. J., Mohapatra, S. K., Horne, W. T., Hontecillas, R. & Bassaganya-Riera, J. The Role of T cell PPAR γ in mice with experimental inflammatory bowel disease. *BMC Gastroenterol.* **10**, 60 (2010).

171. Klotz, L. *et al.* The nuclear receptor PPARγ selectively inhibits Th17 differentiation in a T cell–intrinsic fashion and suppresses CNS autoimmunity. *J. Exp. Med.* **206**, (2009).

172. Feinstein, D. L. *et al.* Peroxisome proliferator-activated receptor-gamma agonists prevent experimental autoimmune encephalomyelitis. *Ann. Neurol.* **51**, 694–702 (2002).

173. Gocke, A. R. *et al.* Transcriptional Modulation of the Immune Response by Peroxisome Proliferator-Activated Receptor- Agonists in Autoimmune Disease. *J. Immunol.* **182**, 4479–4487 (2009).

174. Dunn, S. E. *et al.* Peroxisome proliferator–activated receptor δ limits the expansion of pathogenic Th cells during central nervous system autoimmunity. *J. Exp. Med.* **207**, 1599–1608 (2010).

175. Dumitru, C., Kabat, A. M. & Maloy, K. J. Metabolic Adaptations of CD4+ T Cells in Inflammatory Disease. *Front Immunol* **9**, (2018).

176. Michalek, R. D. *et al.* Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol. Baltim. Md 1950* **186**, 3299–303 (2011).

177. Shi, L. Z. *et al.* HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* **208**, 1367–76 (2011).

178. Xu, T. *et al.* Metabolic control of TH17 and induced Treg cell balance by an epigenetic mechanism. *Nature* **548**, 228–228 (2017).

179. Dang, E. V. *et al.* Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* **146**, 772–784 (2011).

180. Howie, D. *et al.* Foxp3 drives oxidative phosphorylation and protection from lipotoxicity. *JCI Insight* **2**, (2017).

181. Angelin, A. *et al.* Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments. *Cell Metab.* **25**, 1282-1293.e7 (2017).

182. Clever, D. *et al.* Oxygen Sensing by T Cells Establishes an Immunologically Tolerant Metastatic Niche. *Cell* **166**, 1117-1131.e14 (2016).

183. Fu, Z. *et al.* Requirement of Mitochondrial Transcription Factor A in Tissue-Resident Regulatory T Cell Maintenance and Function. *Cell Rep.* **28**, 159-171.e4 (2019).

184. De Rosa, V. *et al.* Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat. Immunol.* **16**, 1174–1184 (2015).

185. Procaccini, C. *et al.* The Proteomic Landscape of Human Ex Vivo Regulatory and Conventional T Cells Reveals Specific Metabolic Requirements. *Immunity* **44**, 406–421 (2016).

186. Gerriets, V. A. *et al.* Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat. Immunol.* **17**, 1459–1466 (2016).

187. Cuadrado, E. *et al.* Proteomic Analyses of Human Regulatory T Cells Reveal Adaptations in Signaling Pathways that Protect Cellular Identity. *Immunity* **48**, 1046-1059.e6 (2018).

188. Wang, Y. *et al.* Histone Deacetylase SIRT1 Negatively Regulates the Differentiation of Interleukin-9-Producing CD4 + T Cells. *Immunity* **44**, 1337–1349 (2016).

189. Delgoffe, G. M. *et al.* The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat. Immunol.* **12**, 295–303 (2011).

190. Lee, K. *et al.* Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* **32**, 743–53 (2010).

191. Yang, J. Q. *et al.* RhoA orchestrates glycolysis for TH2 cell differentiation and allergic airway inflammation. *J. Allergy Clin. Immunol.* **137**, 231-245.e4 (2016).

192. Yang, K. *et al.* T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. *Immunity* **39**, 1043–56 (2013).

193. Zeng, H. *et al.* mTORC1 and mTORC2 Kinase Signaling and Glucose Metabolism Drive Follicular Helper T Cell Differentiation. *Immunity* **45**, 540–554 (2016).

194. Yang, J. *et al.* Critical roles of mTOR Complex 1 and 2 for T follicular helper cell differentiation and germinal center responses. *eLife* **5**, (2016).

195. Peng, M. *et al.* Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* aaf6284–aaf6284 (2016). doi:10.1126/science.aaf6284

196. Qiu, J. *et al.* Acetate Promotes T Cell Effector Function during Glucose Restriction. *Cell Rep.* **27**, 2063-2074.e5 (2019).

197. Gerriets, V. A. *et al.* Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J. Clin. Invest.* **125**, 194–207 (2015).

198. Kaufmann, U. *et al.* Calcium Signaling Controls Pathogenic Th17 Cell-Mediated Inflammation by Regulating Mitochondrial Function. *Cell Metab.* **29**, 1104-1118.e6 (2019).

199. Karmaus, P. W. F. *et al.* Metabolic heterogeneity underlies reciprocal fates of TH17 cell stemness and plasticity. *Nature* **565**, 101–105 (2019).

200. Klysz, D. *et al.* Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci. Signal.* **8**, ra97–ra97 (2015).

201. Johnson, M. O. *et al.* Distinct Regulation of Th17 and Th1 Cell Differentiation by Glutaminase-Dependent Metabolism. *Cell* **175**, 1780-1795.e19 (2018).

202. Bantug, G. R., Galluzzi, L., Kroemer, G. & Hess, C. The spectrum of T cell metabolism in health and disease. *Nat. Rev. Immunol.* **18**, 19–34 (2017).

203. Chang, C.-H. *et al.* Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* **162**, 1229–1241 (2015).

204. Kawalekar, O. U. *et al.* Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. *Immunity* **44**, 380–390 (2016).

205. Menk, A. V. *et al.* 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. *J. Exp. Med.* **215**, 1091–1100 (2018).

206. Ostroukhova, M. *et al.* The role of low-level lactate production in airway inflammation in asthma. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **302**, L300-7 (2012).

207. Glick, G. D. *et al.* Anaplerotic metabolism of alloreactive T cells provides a metabolic approach to treat graft-versus-host disease. *J. Pharmacol. Exp. Ther.* **351**, 298–307 (2014).

208. Nguyen, H. D. *et al.* Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J. Clin. Invest.* **126**, 1337–52 (2016).

209. Gatza, E. *et al.* Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft versus host disease. *Sci. Transl. Med.* **3**, 67ra8 (2011).

210. Wang, C. *et al.* CD5L/AIM Regulates Lipid Biosynthesis and Restrains Th17 Cell Pathogenicity. *Cell* **163**, 1413–1427 (2015).

211. Endo, Y. *et al.* Obesity Drives Th17 Cell Differentiation by Inducing the Lipid Metabolic Kinase, ACC1. *Cell Rep.* **12**, 1042–1055 (2015).

212. Yin, Y. *et al.* Normalization of CD4+ T cell metabolism reverses lupus. *Sci. Transl. Med.* **7**, 274ra18-274ra18 (2015).

213. Keir, M. E., Butte, M. J., Freeman, G. J. & Sharpe, A. H. PD-1 and Its Ligands in Tolerance and Immunity. *Annu. Rev. Immunol.* **26**, 677–704 (2008).

214. Pauken, K. E. & Wherry, E. J. Overcoming T cell exhaustion in infection and cancer. *Trends Immunol.* **36**, 265–276 (2015).

215. Hui, E. *et al.* T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* **355**, 1428–1433 (2017).

216. Yokosuka, T. *et al.* Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *J. Exp. Med.* **209**, 1201–1217 (2012).

217. Francisco, L. M. *et al.* PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J. Exp. Med.* **206**, 3015–29 (2009).

218. Richard V. Parry *et al.* CTLA-4 and PD-1 Receptors Inhibit T-Cell Activation by Distinct Mechanisms. (2005). doi:10.1128/MCB.25.21.9543-9553.2005

219. Wei, S. C., Duffy, C. R. & Allison, J. P. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. *Cancer Discov.* **8**, 1069–1086 (2018).

220. Loke, P. & Allison, J. P. PD-L1 and PD-L2 are differentially regulated by Th1 and Th2 cells. *Proc. Natl. Acad. Sci.* **100**, 5336–5341 (2003).

221. Redpath, S. A. *et al.* Functional specialization of intestinal dendritic cell subsets during Th2 helminth infection in mice. *Eur. J. Immunol.* **48**, 87–98 (2018).

222. Hwang, S., Cobb, D. A., Bhadra, R., Youngblood, B. & Khan, I. A. Blimp-1–mediated CD4 T cell exhaustion causes CD8 T cell dysfunction during chronic toxoplasmosis. *J. Exp. Med.* **213**, 1799–1818 (2016).

223. Sage, P. T. *et al.* Dendritic Cell PD-L1 Limits Autoimmunity and Follicular T Cell Differentiation and Function. *J. Immunol. Baltim. Md 1950* **200**, 2592–2602 (2018).

224. Celada, L. J. *et al.* PD-1 up-regulation on CD4+ T cells promotes pulmonary fibrosis through STAT3-mediated IL-17A and TGF-β1 production. *Sci. Transl. Med.* **10**, eaar8356 (2018).

225. Tkachev, V. *et al.* Programmed Death-1 Controls T Cell Survival by Regulating Oxidative Metabolism. *J. Immunol.* **194**, (2015).

226. Ogando, J. *et al.* PD-1 signaling affects cristae morphology and leads to mitochondrial dysfunction in human CD8+ T lymphocytes. *J. Immunother. Cancer* **7**, 151 (2019).

227. Kouidhi, S., Ben Ayed, F. & Benammar Elgaaied, A. Targeting Tumor Metabolism: A New Challenge to Improve Immunotherapy. *Front. Immunol.* **9**, (2018).

228. Scharping, N. E., Menk, A. V., Whetstone, R. D., Zeng, X. & Delgoffe, G. M. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. *Cancer Immunol. Res.* **5**, 9–16 (2017).

229. Najjar, Y. G. *et al.* Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. *JCI Insight* **4**, (2019).

230. Johnston, C. J. C. *et al.* Cultivation of Heligmosomoides Polygyrus: An Immunomodulatory Nematode Parasite and its Secreted Products. *J. Vis. Exp. JoVE* (2015). doi:10.3791/52412

231. Nobs, S. P. *et al.* PPARγ in dendritic cells and T cells drives pathogenic type-2 effector responses in lung inflammation. *J. Exp. Med.* **214**, 3015–3035 (2017).

232. Matsusue, K. *et al.* Hepatic PPARγ and LXRα independently regulate lipid accumulation in the livers of genetically obese mice. *FEBS Lett.* **588**, 2277–2281 (2014).

233. Byersdorfer, C. A. *et al.* Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood* **122**, 3230–3237 (2013).

234. Pan, Y. *et al.* Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature* **543**, 252–256 (2017).

235. Young, K. E., Flaherty, S., Woodman, K. M., Sharma-Walia, N. & Reynolds, J. M. Fatty acid synthase regulates the pathogenicity of Th17 cells. *J. Leukoc. Biol.* **102**, 1229–1235 (2017).

236. Menk, A. V. *et al.* Early TCR Signaling Induces Rapid Aerobic Glycolysis Enabling Distinct Acute T Cell Effector Functions. *Cell Rep.* **22**, 1509–1521 (2018).

237. Tsai, S. *et al.* Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. *Cell Metab.* **0**, (2018).

238. Bettencourt, I. A. & Powell, J. D. Targeting Metabolism as a Novel Therapeutic Approach to Autoimmunity, Inflammation, and Transplantation. *J. Immunol.* **198**, (2017).

239. Pallett, L. J., Schmidt, N. & Schurich, A. T cell metabolism in chronic viral infection. *Clin. Exp. Immunol.* (2019). doi:10.1111/cei.13308

240. Mohrs, M., Shinkai, K., Mohrs, K. & Locksley, R. M. Analysis of type 2 immunity in vivo with a bicistronic IL-4 reporter. *Immunity* **15**, 303–311 (2001).

241. Perona-Wright, G., Mohrs, K., Mayer, K. D. & Mohrs, M. Differential regulation of IL-4Ralpha expression by antigen versus cytokine stimulation characterizes Th2 progression in vivo. *J. Immunol. Baltim. Md 1950* **184**, 615–23 (2010).

242. Kugler, D. G. *et al.* Systemic toxoplasma infection triggers a long-term defect in the generation and function of naive T lymphocytes. *J. Exp. Med.* **213**, 3041–3056 (2016).

243. Wang, F. *et al.* Glycolytic Stimulation Is Not a Requirement for M2 Macrophage Differentiation. *Cell Metab.* **28**, 463-475.e4 (2018).

244. Ralser, M. *et al.* A catabolic block does not sufficiently explain how 2-deoxy-d-glucose inhibits cell growth. *Proc. Natl. Acad. Sci.* **105**, 17807–17811 (2008).

245. Tan, V. P. & Miyamoto, S. HK2/hexokinase-II integrates glycolysis and autophagy to confer cellular protection. *Autophagy* **11**, 963–4 (2015).

246. Kabat, A. M. *et al.* The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. *eLife* **5**, e12444–e12444 (2016).

247. Waickman, A. T. & Powell, J. D. mTOR, metabolism, and the regulation of T-cell differentiation and function. *Immunol. Rev.* **249**, 43–58 (2012).

248. Ricardo-Gonzalez, R. R. *et al.* IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 22617–22 (2010).

249. Wu, D. *et al.* Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* **332**, 243–7 (2011).

250. Constant, S., Pfeiffer, C., Woodard, A., Pasqualini, T. & Bottomly, K. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J. Exp. Med.* **182**, 1591–6 (1995).

251. Tao, X., Constant, S., Jorritsma, P. & Bottomly, K. Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4+ T cell differentiation. *J. Immunol. Baltim. Md 1950* **159**, 5956–63 (1997).

252. Tubo, N. J. & Jenkins, M. K. TCR signal quantity and quality in CD4+ T cell differentiation. *Trends Immunol.* **35**, 591–596 (2014).

253. van Panhuys, N., Klauschen, F. & Germain, R. N. T-cell-receptor-dependent signal intensity dominantly controls CD4(+) T cell polarization In Vivo. *Immunity* **41**, 63–74 (2014).

254. Scheu, S. *et al.* Activation of the integrated stress response during T helper cell differentiation. *Nat. Immunol.* **7**, 644–651 (2006).

255. Casolaro, V. *et al.* Posttranscriptional regulation of IL-13 in T cells: Role of the RNA-binding protein HuR. *J. Allergy Clin. Immunol.* **121**, 853–9.e4 (2008).

256. Phan, A. T., Goldrath, A. W. & Glass, C. K. Metabolic and epigenetic coordination of T cell and Macrophage immunity. *Immunity* **46**, 714–729 (2017).

257. Baixauli, F. *et al.* Mitochondrial respiration controls lysosomal function during inflammatory t cell responses. *Cell Metab.* **22**, 485–498 (2015).

258. Chen, X. *et al.* Early-life undernutrition reprograms CD4+ T-cell glycolysis and epigenetics to facilitate asthma. *J. Allergy Clin. Immunol.* **143**, 2038-2051.e12 (2019).

259. Allen, J. E. & Maizels, R. M. Diversity and dialogue in immunity to helminths. *Nat. Rev. Immunol.* **11**, 375–88 (2011).

260. Taylor, M. D., van der Werf, N. & Maizels, R. M. T cells in helminth infection: the regulators and the regulated. *Trends Immunol.* **33**, 181–189 (2012).

261. van der Werf, N., Redpath, S. A., Azuma, M., Yagita, H. & Taylor, M. D. Th2 cell-intrinsic hypo-responsiveness determines susceptibility to helminth infection. *PLOS Pathog.* **9**, e1003215–e1003215 (2013).

262. Sanchez, E. L. & Lagunoff, M. Viral activation of cellular metabolism. *Virology* **479–480**, 609–618 (2015).

263. Duette, G. *et al.* Induction of HIF-1α by HIV-1 Infection in CD4+ T Cells Promotes Viral Replication and Drives Extracellular Vesicle-Mediated Inflammation. *mBio* **9**, e00757-18 (2018).

264. Bridgette M Cumming, Kelvin W Addicott, John H Adamson & Adrie JC Steyn. Mycobacterium tuberculosis induces decelerated bioenergetic metabolism in human macrophages. (2018). doi:doi:10.7554/eLife.39169

265. Huang, L., Nazarova, E. V., Tan, S., Liu, Y. & Russell, D. G. Growth of Mycobacterium tuberculosis in vivo segregates with host macrophage metabolism and ontogeny. *J. Exp. Med.* **215**, 1135–1152 (2018).

266. Ip, W. K. E., Hoshi, N., Shouval, D. S., Snapper, S. & Medzhitov, R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science* **356**, (2017).

267. Hewitson, J. P. *et al.* Proteomic analysis of secretory products from the model gastrointestinal nematode Heligmosomoides polygyrus reveals dominance of Venom Allergen-Like (VAL) proteins. *J. Proteomics* **74**, 1573–1594 (2011).

268. Pollizzi, K. N., Waickman, A. T., Patel, C. H., Sun, I. H. & Powell, J. D. Cellular size as a means of tracking mTOR activity and cell fate of CD4+ T cells upon antigen recognition. *PLoS ONE* **10**, e0121710–e0121710 (2015).

269. Delisle, J. S. *et al.* The TGF-β-Smad3 pathway inhibits CD28-dependent cell growth and proliferation of CD4 T cells. *Genes Immun.* **14**, 115–126 (2013).

270. Grunwell, J. R. *et al.* TGF-β1 Suppresses the Type I IFN Response and Induces Mitochondrial Dysfunction in Alveolar Macrophages. *J. Immunol. Baltim. Md 1950* **200**, 2115–2128 (2018).

271. Viel, S. *et al.* TGF- inhibits the activation and functions of NK cells by repressing the mTOR pathway. *Sci. Signal.* **9**, ra19–ra19 (2016).

272. Johnston, C. J. C. *et al.* A structurally distinct TGF-β mimic from an intestinal helminth parasite potently induces regulatory T cells. *Nat. Commun.* **8**, 1741–1741 (2017).

273. Bravo-Santano, N. *et al.* Intracellular Staphylococcus aureus Modulates Host Central Carbon Metabolism To Activate Autophagy. *mSphere* **3**, (2018).

274. Vignali, D. A. A., Collison, L. W. & Workman, C. J. How regulatory T cells work. *Nat. Rev. Immunol.* **8**, 523–532 (2008).

275. Priyadharshini, B. *et al.* Cutting Edge: TGF-β and Phosphatidylinositol 3-Kinase Signals Modulate Distinct Metabolism of Regulatory T Cell Subsets. *J. Immunol. Baltim. Md 1950* **201**, 2215–2219 (2018).

276. McSorley, H. J. *et al.* Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus. *Eur. J. Immunol.* **42**, 2667–2682 (2012).

277. Bao, K. & Reinhardt, R. L. The differential expression of IL-4 and IL-13 and its impact on type-2 Immunity. *Cytokine* **75**, 25–37 (2015).

278. Yang, D., Han, Z. & Oppenheim, J. J. ALARMINS AND IMMUNITY. *Immunol. Rev.* **280**, 41–56 (2017).

279. Peine, M., Marek, R. M. & Löhning, M. IL-33 in T Cell Differentiation, Function, and Immune Homeostasis. *Trends Immunol.* **37**, 321–333 (2016).

280. Guo, L. *et al.* IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc. Natl. Acad. Sci.* **106**, 13463–13468 (2009).

281. Endo, Y. *et al.* The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity* **42**, 294–308 (2015).

282. Townsend, M. J., Fallon, P. G., Matthews, D. J., Jolin, H. E. & McKenzie, A. N. J. T1/St2-Deficient Mice Demonstrate the Importance of T1/St2 in Developing Primary T Helper Cell Type 2 Responses. *J. Exp. Med.* **191**, 1069–1076 (2000).

283. Humphreys, N. E., Xu, D., Hepworth, M. R., Liew, F. Y. & Grencis, R. K. IL-33, a Potent Inducer of Adaptive Immunity to Intestinal Nematodes. *J. Immunol.* **180**, 2443–2449 (2008).

284. Hung, L.-Y. *et al.* IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 282–7 (2013).

285. Wilhelm, C. *et al.* Critical role of fatty acid metabolism in ILC2-mediated barrier protection during malnutrition and helminth infection. *J. Exp. Med.* **213**, 1409–1418 (2016).

286. Vats, D. *et al.* Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab.* **4**, 13–24 (2006).

287. Chen, T. *et al.* PPAR-γ promotes type 2 immune responses in allergy and nematode infection. *Sci. Immunol.* **2**, (2017).

288. Schiering, C. *et al.* The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* **513**, 564–568 (2014).

289. Alvarez, F. *et al.* The alarmins IL-1 and IL-33 differentially regulate the functional specialisation of Foxp3 + regulatory T cells during mucosal inflammation. *Mucosal Immunol.* **12**, 746 (2019).

290. Dunn, S. E. *et al.* Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. *J. Exp. Med.* **204**, 321–30 (2007).

291. Zhang, M. A. *et al.* Peroxisome proliferator-activated receptor (PPAR)α and -γ regulate IFNγ and IL-17A production by human T cells in a sex-specific way. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 9505–10 (2012).

292. Robinette, M. L. *et al.* Transcriptional Programs Define Molecular Characteristics of Innate Lymphoid Cell Classes and Subsets. *Nat. Immunol.* **16**, 306–317 (2015).

293. Szanto, A. *et al.* STAT6 Transcription Factor Is a Facilitator of the Nuclear Receptor PPARγ-Regulated Gene Expression in Macrophages and Dendritic Cells. *Immunity* **33**, 699–712 (2010).

294. Perona-Wright, G., Mohrs, K. & Mohrs, M. Sustained signaling by canonical helper T cell cytokines throughout the reactive lymph node. *Nat. Immunol.* **11**, 520–6 (2010).

295. Cheng, Y. *et al.* Trichinella spiralis Infection Mitigates Collagen-Induced Arthritis via Programmed Death 1-Mediated Immunomodulation. *Front. Immunol.* **9**, (2018).

296. Oflazoglu, E. *et al.* Paradoxical role of programmed death-1 ligand 2 in Th2 immune responses in vitro and in a mouse asthma model in vivo. *Eur. J. Immunol.* **34**, 3326–3336 (2004).

297. Zhou, S. *et al.* Blockade of PD-1 Signaling Enhances Th2 Cell Responses and Aggravates Liver Immunopathology in Mice with Schistosomiasis japonica. *PLoS Negl. Trop. Dis.* **10**, e0005094–e0005094 (2016).

298. Huber, S. *et al.* Alternatively activated macrophages inhibit T-cell proliferation by Stat6-dependent expression of PD-L2. *Blood* **116**, 3311–20 (2010).

299. Svedberg, F. R. *et al.* The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. *Nat. Immunol.* 1 (2019). doi:10.1038/s41590-019-0352-y

300. Hams, E. *et al.* Blockade of B7-H1 (programmed death ligand 1) enhances humoral immunity by positively regulating the generation of T follicular helper cells. *J. Immunol. Baltim. Md 1950* **186**, 5648–55 (2011).

301. Good-Jacobson, K. L. *et al.* PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat. Immunol.* **11**, 535–542 (2010).

302. Khan, A. R. *et al.* PD-L1^{hi} B cells are critical regulators of humoral immunity. *Nat. Commun.* **6**, 5997 (2015).

303. Kusam, S., Toney, L. M., Sato, H. & Dent, A. L. Inhibition of Th2 Differentiation and GATA-3 Expression by BCL-6. *J. Immunol.* **170**, 2435–2441 (2003).

304. Lüthje, K. *et al.* The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. *Nat. Immunol.* **13**, 491–498 (2012).

305. Meisel, C. *et al.* Regulation and Function of T1/ST2 Expression on CD4+ T Cells: Induction of Type 2 Cytokine Production by T1/ST2 Cross-Linking. *J. Immunol.* **166**, 3143–3150 (2001).

306. Löhning, M. *et al.* T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6930–6935 (1998).

307. Tuncel, J., Benoist, C. & Mathis, D. T cell anergy in perinatal mice is promoted by T reg cells and prevented by IL-33. *J. Exp. Med.* **216**, 1328–1344 (2019).

308. Li, Q. *et al.* E3 Ligase VHL Promotes Group 2 Innate Lymphoid Cell Maturation and Function via Glycolysis Inhibition and Induction of Interleukin-33 Receptor. *Immunity* **48**, 258-270.e5 (2018).

309. Cho, S. H. *et al.* Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature* **537**, 234–238 (2016).

310. Ricciardi, S. *et al.* The Translational Machinery of Human CD4+ T Cells Is Poised for Activation and Controls the Switch from Quiescence to Metabolic Remodeling. *Cell Metab.* **28**, 895-906.e5 (2018).

311. Webster, H. C., Andrusaite, A. T., Shergold, A. L., Milling, S. W. F. & Perona-Wright, G. Isolation and functional characterisation of lamina propria leukocytes from helminth-infected, murine small intestine. *bioRxiv* 750786 (2019). doi:10.1101/750786

312. Huang, S. C.-C. *et al.* Fatty Acid Oxidation Is Essential for Egg Production by the Parasitic Flatworm Schistosoma mansoni. *PLOS Pathog.* **8**, e1002996–e1002996 (2012).

313. Asojo, O. A. *et al.* Heligmosomoides polygyrus Venom Allergen-like Protein-4 (HpVAL-4) is a sterol binding protein. *Int. J. Parasitol.* **48**, 359–369 (2018).

314. Johnson, M. O., Siska, P. J., Contreras, D. C. & Rathmell, J. C. Nutrients and the microenvironment to feed a T cell army. *Semin. Immunol.* **28**, 505–513 (2016).

315. Alwarawrah, Y., Kiernan, K. & MacIver, N. J. Changes in Nutritional Status Impact Immune Cell Metabolism and Function. *Front. Immunol.* **9**, (2018).

316. Walls, J., Sinclair, L. & Finlay, D. Nutrient sensing, signal transduction and immune responses. *Semin. Immunol.* **28**, 396–407 (2016).

317. Caputa, G., Castoldi, A. & Pearce, E. J. Metabolic adaptations of tissue-resident immune cells. *Nat. Immunol.* **20**, 793–801 (2019).

318. Garnett, J. P., Baker, E. H. & Baines, D. L. Sweet talk: insights into the nature and importance of glucose transport in lung epithelium. *Eur. Respir. J.* **40**, 1269–1276 (2012).

319. Gill, S. K. *et al.* Increased airway glucose increases airway bacterial load in hyperglycaemia. *Sci. Rep.* **6**, 27636 (2016).

320. Mallia, P. *et al.* Role of airway glucose in bacterial infections in patients with chronic obstructive pulmonary disease. *J. Allergy Clin. Immunol.* **142**, 815-823.e6 (2018).

321. Chroneos, Z. C., Sever-Chroneos, Z. & Shepherd, V. L. Pulmonary Surfactant: An Immunological Perspective. *Cell. Physiol. Biochem.* **25**, 13–26 (2009).

322. Cipolletta, D. *et al.* PPAR-? is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* **486**, 549–53 (2012).

323. Vasanthakumar, A. *et al.* The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue–resident regulatory T cells. *Nat. Immunol.* **16**, 276–285 (2015).

324. Chen, F. *et al.* An essential role for the Th2-type response in limiting tissue damage during helminth infection. *Nat. Med.* **18**, 260–266 (2012).

325. Abbott, R. K. *et al.* Germinal Center Hypoxia Potentiates Immunoglobulin Class Switch Recombination. *J. Immunol.* **197**, 4014–4020 (2016).

326. Finlay, D. K. *et al.* PDK1 regulation of mTOR and hypoxia-inducible factor 1 integrate metabolism and migration of CD8+ T cells. *J. Exp. Med.* **209**, (2012).

327. Xu, Y. *et al.* Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. *J. Clin. Invest.* **126**, 2678–2688 (2016).

328. Sedhom, M. A. K. *et al.* Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice. *Gut* **62**, 1714–1723 (2013).

329. Pushparaj, P. N. *et al.* Interleukin-33 exacerbates acute colitis via interleukin-4 in mice. *Immunology* **140**, 70–77 (2013).

330. Filbey, K. J. *et al.* Intestinal helminth infection promotes IL-5- and CD4 + T cell-dependent immunity in the lung against migrating parasites. *Mucosal Immunol.* **12**, 352 (2019).

331. Krishnamoorthy, V. *et al.* The IRF4 Gene Regulatory Module Functions as a Read-Write Integrator to Dynamically Coordinate T Helper Cell Fate. *Immunity* **47**, 481-497.e7 (2017).

332. Kolodin, D. *et al.* Antigen- and Cytokine-Driven Accumulation of Regulatory T Cells in Visceral Adipose Tissue of Lean Mice. *Cell Metab.* **21**, 543–557 (2015).

333. Chawla, A., Nguyen, K. D. & Goh, Y. P. S. Macrophage-mediated inflammation in metabolic disease. *Nat. Rev. Immunol.* **11**, 738–749 (2011).

334. McGuinness, O. P. Defective Glucose Homeostasis During Infection. *Annu. Rev. Nutr.* **25**, 9–35 (2005).

335. Wiria, A. E. *et al.* Infection with Soil-Transmitted Helminths Is Associated with Increased Insulin Sensitivity. *PloS One* **10**, e0127746 (2015).

336. Hussaarts, L. *et al.* Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* **29**, 3027–39 (2015).

337. La Flamme, A. C. *et al.* Chronic exposure to schistosome eggs reduces serum cholesterol but has no effect on atherosclerotic lesion development. *Parasite Immunol.* **29**, 259–266 (2007).

338. Shimokawa, C. *et al.* Suppression of Obesity by an Intestinal Helminth through Interactions with Intestinal Microbiota. *Infect. Immun.* **87**, e00042-19 (2019).

339. Su, C. wen *et al.* Helminth infection protects against high fat diet-induced obesity via induction of alternatively activated macrophages. *Sci. Rep.* **8**, 4607 (2018).

340. Wong, T. *et al.* Divergent Metabolic Adaptations to Intestinal Parasitic Nematode Infection in Mice Susceptible or Resistant to Obesity. *Gastroenterology* **133**, 1979–1979 (2007).