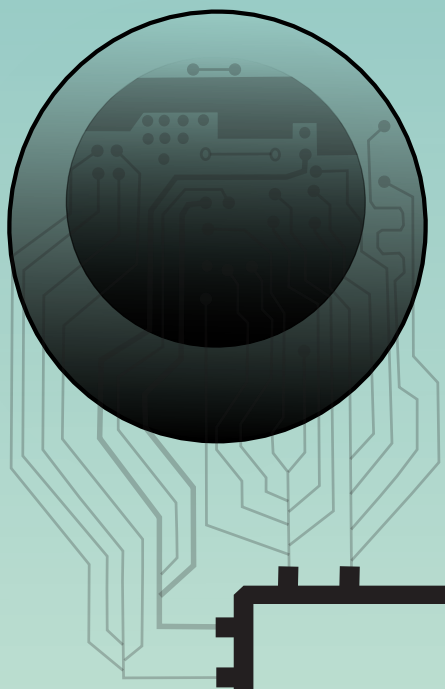
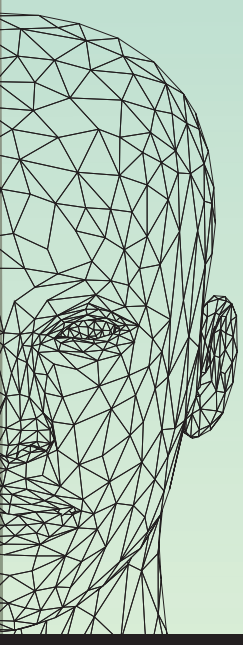
Turun yliopisto
University of Turku



NOVEL REGULATORS OF T HELPER CELL DIFFERENTIATION CONTRIBUTE TO AUTOIMMUNE INFLAMMATION

Imran Mohammad



TURUN YLIOPISTON JULKAISUJA - ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1383 | Medica - Odontologica | Turku 2018

Turun yliopisto
University of Turku

NOVEL REGULATORS OF T HELPER CELL DIFFERENTIATION CONTRIBUTE TO AUTOIMMUNE INFLAMMATION

Imran Mohammad

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1383 | Medica - Odontologica | Turku 2018

University of Turku

Faculty of Medicine
Medical Microbiology and Immunology,
Turku Doctoral Programme of Molecular Medicine
Turku Centre for Biotechnology, University of Turku and Åbo Akademi University

Supervised by

Docent Zhi Chen, M.D., Ph.D.
Turku Centre for Biotechnology
University of Turku and Åbo Akademi University
Turku, Finland

Reviewed by

Professor Marko Pesu, M.D., Ph.D.
Faculty of Medicine and Life Sciences
University of Tampere
Tampere, Finland

Docent John Andersson, Ph.D.
Karolinska institutet
Karolinska University Hospital
Stockholm, Sweden

Opponent

Professor Mika Rämet, M.D., Ph.D.
Faculty of Medicine
University of Oulu
Oulu, Finland

Cover by Imran Mohammad, human vector artwork adapted from https://publicdomainvectors.org/en/free-clipart/Wire-frame-human-head/52196.html

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-7394-1 (PRINT)
ISBN 978-951-29-7395-8 (PDF)
ISSN 0355-9483 (PRINT)
ISSN 2343-3213 (PDF)
Painosalama Oy – Turku, Finland 2018

ABSTRACT

Imran Mohammad

Novel regulators of T helper cell differentiation contribute to autoimmune inflammation

University of Turku, Faculty of Medicine, Medical Microbiology and Immunology
Turku Doctoral Programme of Molecular Medicine
Turku Centre for Biotechnology, University of Turku and Åbo Akademi University

T helper cells are critical components of adaptive immune system contributing to self/ nonself-recognition, pathogen elimination and pathogenesis of immune-associated diseases.

Understanding the underlying mechanisms and signaling changes associated with T cell differentiation and autoimmune inflammatory disease processes remains far from being complete. The studies presented in this PhD thesis were aimed to investigate the role of a suspected/known contributor to autoimmune inflammation and to identify novel modulators that regulate Th17 and iTreg cell differentiation.

Autoimmune diseases show greater predominance in females than in males and the underlying mechanisms of this are not well understood. In this study, the role of estrogen receptor alpha (ERα) in the development of autoimmune T cell responses has been addressed. T cell specific ERα depleted mice were induced to develop a T cell mediate autoimmune disease. The results indicated that ERα contributes to autoimmune inflammation, T cell activation, proliferation, and survival. Additionally, in regulatory T cells the ERα protein was observed to regulate the expression of Foxp3. The results of this study provide insight into understanding the role of estrogen receptor in autoimmune diseases and also provide implications for the development of novel therapeutics. To further identify novel regulators of Th17 and iTreg cell differentiation, a label-free quantitative proteomics approach was applied to profiling the protein expression changes from whole Th17 and iTreg cells. Selected proteins that showed expression changes during Th17 and iTreg cell differentiation were validated. Proteomic and transcriptomic data was combined to identify the proteins that are differentially regulated in Th17 or iTreg cells only at protein level. The work presented in this thesis provides an insight into the proteome of important T helper cell subsets with further implications for further development of novel therapies to treat autoimmune diseases.

Keywords: T helper cell differentiation, Th17 cells, iTreg cells, Proteomics, Estrogen receptor, Colitis, Autoimmune diseases, Sex bias

TIIVISTELMÄ

Imran Mohammad

T-solujen erilaistumiseen vaikuttavat uudet säätelijät osallistuvat autoimmuunitulehdukseen

Turun yliopisto Lääketieteellinen tiedekunta, Lääketieteellinen mikrobiologia ja immunologia
Turun molekyylilääketieteen tohtoriohjelma
Turun Biotekniikan keskus, Turun yliopisto ja Åbo Akademi

T-auttajasolut ovat olennainen osa hankittua immuunijärjestelmää, jossa ne toimivat mukana oma- ja vierasperäisten tekijöiden tunnistamisessa, taudinaiheuttajien tuhoamisessa ja tiettyjen sairauksien immuunireaktioissa. T-solujen erilaistumiseen ja autoimmuunitulehdussairauksien kehittymiseen vaikuttavien mekanismien ja signaalireittien muutosten ymmärtäminen on yhä kaukana kokonaisvaltaisesta kuvasta. Kyseisen tohtorikoulutustutkimuksen tavoitteena oli tutkia autoimmuunitulehdukseen vaikuttavaa tekijää sekä tunnistaa uusia proteiineja, jotka säätelevät auttaja-T-17-solujen (Th17) ja indusoitujen säätelijä-T-solujen (iTreg) erilaistumista.

Autoimmuunisairaudet ovat yleisempiä naisilla kuin miehillä ja sen perustana olevat mekanismit eivät ole selvitetty. Ensimmäisen tutkimuksen kohteena oli estrogeenireseptori-alfan (ERα) rooli T-soluvälitteisten autoimmuunisairauksien kehittymisessä. ERα-puutteiset T-solut hiiressä altistettiin T-soluvälitteisille autoimmuunisairauksille. Tulokset osoittavat että ERα vaikuttaa tulehdukseen sekä solujen aktivaatioon, jakautumiseen ja selviytymiseen. ERα-puutteiset T-solut osoittavat Foxp3 proteiinin kohonnutta ilmenemistä. Tulokset selvittävät hormonireseptorien roolia autoimmuunisairauksissa sekä niiden käyttömahdollisuutta uusien hoitomenetelmien kehityksessä.

Uusien Th17- ja iTreg-solujen erilaistumiseen vaikuttavien säätelijöiden tunnistamiseksi käytettiin merkkiaineetonta ja kvantitatiivista proteomiikkamenetelmää. Proteiinien ilmenemismuutokset profiloitiin Th17- ja iTreg-solujen eri osista ja valitut proteiinit varmistettiin lisäkokeilla. Proteomiikka ja transkriptomiikkatuloksia verrattiin keskenään näissä eri tavoin käyttäytyvien solun proteiinien tunnistamiseksi. Tutkimus tuotti arvokasta aineistoa uusien hoitomenetelmien kehitykseen autoimmuunisairauksissa.

Avainsanat: Auttaja-T-solujen erilaistuminen, Th17-solut, iTreg-solut, proteomiikka, estrogeenireseptori, koliitti, autoimmuunisairaudet, sukupuolivaikutus

CONTENTS

ABBREVIATIONS

ATRA	All-Trans retinoic acid
AHR	aryl hydrocarbon receptor
APC	antigen presenting cell
BCL6	B-cell CLL/lymphoma 6
BCR	B cell receptor
β-ME	β-mercaptoethanol
BSA	bovine serum albumin
CD	cluster of differentiation
cDC	conventional DC
cDNA	complementary DNA
ChIP	chromatin immunoprecipitation
CNS	conserved non-coding sequence
CRISPR	clustered, regularly interspaced short palindromic repeats
Ct	cycle of threshold
DC	dendritic cell
DHS	DNase1 hypersensitive sites
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E2	17β-Estradiol
EAE	experimental autoimmune encephalomyelitis
ER	Estrogen receptor
FAO	Fattyacid oxidation
FCS	fetal calf serum
FDR	false discovery rate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GATA3	GATA binding protein 3
GC	germinal centre
HIES	hyper-immunoglobulin E syndrome, Job's syndrome
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRP	horseradish peroxidase
IAA	Iodoacetamide
IBD	inflammatory bowel disease
ICOS	inducible T-cell co-stimulator
IFN-α	interferon alpha
Ig	immunoglobulin

IL	interleukin
ILC	innate lymphoid cell
IRF	interferon regulatory factor
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LTi	lymphoid tissue inducer cell
MAPK	mitogen activated protein kinase
miRNA	micro RNA
mRNA	messenger RNA
MS	multiple sclerosis
mTEC	thymic epithelial cells of medulla
NK	natural killer cell
Nt	nucleotide
PBS	phosphate buffered saline
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell
RA	rheumatoid arthritis
RNA	ribonucleic acid
RNAi	RNA interference
RORC	RAR-related orphan receptor C
RPMI	Roswell park memorial institute medium
rRNA	ribosomal RNA
RT-qPCR	reverse transcriptase quantitative polymerase chain reaction
siRNA	small interfering RNA
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
STAT	signal transducer and activator of transcription
Tc	cytotoxic T cell
TCR	T cell receptor
TFs	transcription factors
Tfh	T follicular helper cell
TFBS	transcription factor binding site
TGF	transforming growth factor
Th	T helper cell
TNF-α	tumor necrosis factor alpha
Treg	T regulatory cell
TSS	transcription starting site
UC	ulcerative colitis

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on following original publications.

I. Mohammad, Imran*, Inna Starskaia*, Tamas Nagy, Jitao Guo, Emrah Yatkin, Kalervo Väänänen, Wendy T. Watford, and Zhi Chen (2018). Estrogen Receptor α Contributes to T Cell–mediated Autoimmune Inflammation by Promoting T Cell Activation and Proliferation. Science Signaling 11, 526. eaap9415. (* shared first authorship)

II. Mohammad, Imran, Kari Nousiainen, Santosh D. Bhosale, Inna Starskaia, Robert Moulder, Anne Rokka, Fang Cheng, Ponnuswamy Mohanasundaram, John E. Eriksson, David R. Goodlett, Harri Lähdesmäki and Zhi Chen (2018). Quantitative proteomic characterization and comparison of T helper 17 and induced regulatory T cells. PLoS Biology. 16, e2004194.

1 INTRODUCTION

The adaptive immune system orchestrates specific immune responses, such as protection against pathogens and self-tolerance. The dysregulation of this system creates a situation where the body starts attacking its own cells leading to autoimmunity. T helper cells such as T helper 17 (Th17) and regulatory T (iTreg) cells are active players in autoimmune inflammation and immune tolerance, respectively.

The higher prevalence of autoimmune diseases in females has been a long standing question. Although the estrogen hormone is observed to play a role, the molecular basis of the disease under the influence of estrogen receptor alpha is not clearly understood. To address this question, the work conducted in this thesis includes evaluation of the role of estrogen receptor alpha in the context of autoimmunity. This was achieved by subjecting T cell specific ERα deficient mice with T cell mediated colitis (Publication I).

Multiple studies have extensively characterized the transcriptional and epigenetic regulation in Th17 and iTreg cells. However, no study to date has reported the proteomic profile of Th17 cells. In this thesis, we performed proteomic characterization of Th17 and iTreg cells to identify the novel regulators of differentiation of proteins that define the lineage commitment (Publication II). We further validated the function of vimentin in iTreg cells to explore the findings of our study.

The outcome and results of the thesis helps in targeting the protein molecules for immunotherapy, to study immune responses in hormonal therapy, and to design novel strategies in treating autoimmune disease.

2 REVIEW OF THE LITERATURE

2.1 The Immune system in a nutshell

The human body is under siege from a range of pathogens all the time. The immune system is comprised of a varied arsenal of weapons (biological structures) that constantly protect the host from external and internal agents. However, not all external agents are pathogenic, e.g. certain types of microbiota are beneficial to the host and a symbiotic association is observed within the immune compartment. The microbiota in the gut degrade polysaccharides and dietary substances resulting in increased digestive efficiency for the host and in turn gain a steady supply of nutrients (Hooper et al., 2012). Furthermore, short-chain fatty acids (SCFA) derived from colonic microbiota enhance the induction of murine regulatory T lymphocytes (Arpaia et al., 2013). In the perspective of evolution, all the organisms have at least one form of defense mechanism to protect them from pathogens. CRISPR-Cas (clustered, regularly interspaced short palindromic repeats/CRISPR associated) is the adaptive and heritable immune system against viruses observed in prokaryotes. In the CRISPR-Cas system, bacteria capture sequences of DNA from invading viruses and incorporate sequences into the CRISPR locus. The CRISPR locus enables the bacteria to remember/recognize the viruses and upon re-infection, the bacteria produce RNA segments (small antisense RNAs, known as crRNAs) from the CRISPR locus. The bacteria then uses crRNA-guided Cas nucleases (Cas9) to cleave the invading viral DNA to clear the infection (Barrangou and Marraffini, 2014; Marraffini and Sontheimer, 2010). Discrimination of self from non-self components is one of the basic functions of immune system. Protozoans, tunicates, and invertebrates show the ability to distinguish self from non-self. Sponges accept grafts from their own colony but reject tissue grafts from different colonies indicating that the immune protection is observed at the level of evolution (Buchmann, 2014; Loker et al., 2004).

Based on its modes of action, the immune system is classified into innate (non-specific) and adaptive (specific immunity) (**Figure 1**). Both systems work together to eliminate and restrict the proliferation of pathogens from the human body. The systems

also recognize abnormal cells that develop into cancer (Alberts et al., 2002; Janeway et al., 2001).

2.1.1 Innate immunity

The innate immune system provides a non-specific response (the first line of defense) and includes physical barriers, chemical barriers and immune cells. Namely, the physical barriers include the skin and the chemical barriers the low pH of the stomach, which inhibits the growth of pathogens. Immune cells, such as macrophages, neutrophils, monocytes, NK cells and dendritic cells (Figure 1) are constituents of the innate immune system. Macrophages and neutrophils are responsible for inflammatory responses and phagocytosis (Janeway et al., 2001). Macrophages are phagocytic cells that use toll-like receptors (TLRs) to recognize molecules on the microbial surface (Greenberg and Grinstein, 2002). TLRs specifically bind to lipopolysaccharide (LPS) on Gram-negative bacteria, teichoic acids on Gram-positive bacteria, RNA, DNA and extracellular proteins (Schwandner et al., 1999). Macrophages secrete a variety of mediators that recruit neutrophils to the site of inflammation. Neutrophils are the second major class of phagocytic cells. These cells are short-lived and are among the first cells to provide innate immunity against infectious agents (Janeway et al., 2001). In addition, macrophages and neutrophils also evoke the complement system. The complement system is a group of soluble serum proteins, induces lysis of the invading microorganism by aiding in the phagocytosis of the pathogen and forming a pore in the cell membrane called membrane attack complex (MAC) resulting in clearance of pathogen (Merle et al., 2015). Mast cells are long-lived cells with an important role in antigen presentation, phagocytosis and specialized in amplifying or suppressing innate or acquired immune responses (Urb and Sheppard, 2012). Mast cells, basophils, and eosinophils are critical players in allergic responses and asthma pathogenesis, show overlapping functions in the induction of allergy and in the release of inflammatory mediators such as histamine, proteases, chemotactic factors, and cytokines (Stone et al., 2010). Natural killer (NK) cells constantly patrol the body and eliminate cancer cells or virus-infected cells (Wu and Lanier, 2003). Innate lymphoid cells (ILCs) are an emerging group of innate cells that are developmentally closely related to NK cells. ILCs are weakly cytotoxic, whereas NK cells secrete perforin and granzyme to show

cytotoxic activity (Colonna, 2018). ILCs cells lack B or T cell receptors show similarity in the pattern of cytokine production with T helper lymphocytes- a class of adaptive immune system (Spits et al., 2013). Dendritic cells (DC) form the third class of phagocytic cells. DCs are a unique type of antigen-presenting cells (APCs) with the ability to evoke primary immune responses and establish the immunological memory (Banchereau and Steinman, 1998). DCs are sub-classified into plasmacytoid dendritic cells (pDCs) and conventional or classical dendritic cells (cDCs). pDCs are a small subset accumulated mainly in blood and lymphoid tissues. cDCs populate lymphoid and non-lymphoid tissues and express high levels of MHC class II. The main function of these cells is to process antigens and present them to the T cells to initiate the next level of an adaptive immune response (Steinman, 1991; Worbs et al., 2017).

2.1.2 Acquired immunity

The adaptive immune system involves highly specialized and systemic cells for clearing pathogens. There are two types of adaptive immunity: humoral immunity mediated by antibodies produced from B lymphocytes, and the cell-mediated immunity elicited by T lymphocytes.

After infection, activated B cells differentiate either into plasma cells to produce antibodies or become memory cells that provide the responses against secondary infection. During infection, dendritic cells present antigen to T lymphocytes. The activated T cell then responds against the pathogen either by secreting cytokine mediators or by invoking B lymphocyte responses. Memory B cells and memory T cells form the immunological memory from which a more robust and rapid immune response is elicited to a second exposure with the same antigen. This system is highly adaptable with response against a wide range of pathogens because of the broad diversity of immune cell surface receptors. The mechanisms of somatic hypermutation and V(D)J recombination allow a small amount of genetic material to encode a large number of different antigen receptors (Janeway et al., 2001).

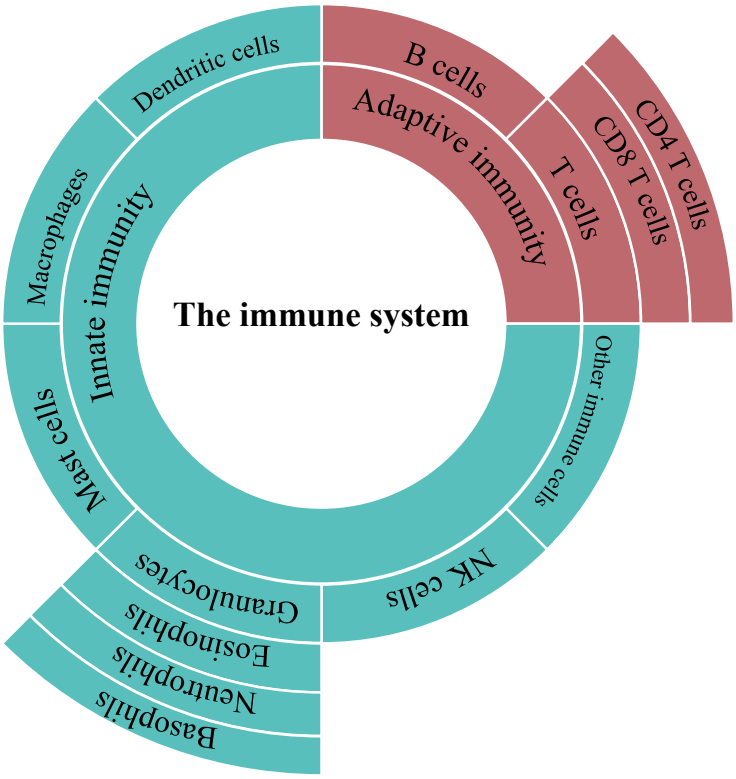


Figure 1: Plot showing the hierarchical classification of cells involved in the Immune system. The size of the component shown here is based on the number of sub divisions in each component and does not reflect the magnitude of their contribution to the immune system.

B cells and T cells are distinguished on the basis of the presence of their antigen surface receptors, i.e. BCR (B cell receptor) and TCR (T cell receptor). T lymphocytes are further subclassified into $CD4^+$ T helper cells and $CD8^+$ T cytotoxic cells. MHC class II molecules on APC interact with CD4 receptors and MHC class I interact with CD8 receptors. $CD4^+$ T helper cells secrete specific cytokines and stimulate the cells such as B cells for antibody production and macrophages in killing the phagocytosed pathogen. $CD8^+$ T cytotoxic cells kill the cells infected with viruses and intracellular pathogens (Janeway et al., 2001). $CD4^+$ T helper cells undergo T cell activation and differentiation to transform into an effector T cell.

2.2 T helper cell activation

T cell activation initiates complex signaling events that determine cell survival, proliferation, differentiation, and cytokine production. With sufficient interaction between T cell and antigen-presenting cell (APC), reorganization of the cytoskeleton and accumulation of a cluster of signaling molecules leads to the formation of the supramolecular activation complex (SMAC) in T cells. This complex consists of a center ring of signaling molecules called cSMAC, and a peripheral ring of adhesion molecules, termed pSMAC (Monks et al., 1998). Three signals are required for full activation of a T cell: TCR stimulation by antigen-MHC (signal one), simultaneous engagement of CD28 co-stimulatory molecule (signal two) and cytokine (signal three) (Corthay, 2006; Curtsinger et al., 1999).

2.2.1 Signal One

TCR triggering causes the phosphorylation of TCR/CD3 cytosolic component immunoreceptor tyrosine-based activation motifs (ITAMs) by protein tyrosine kinases Lck and Fyn. Zeta-chain-associated protein kinase (Zap-70) is recruited to ITAMs and phosphorylates linker for activation of T cells (LAT)(Zhang et al., 1998) and SH2-domain –containing leukocyte protein (SLP-76) (Wardenburg et al., 1996) leading to the downstream events of LAT signalosome. Multiple signaling proteins such as guanine nucleotide exchange factor (GEF) Vav1, growth factor receptor-bound protein 2 (Grb2), adaptor proteins NCK, GRB2-related adapter protein 2 (Gads) and an inducible T cell kinase (Itk) are involved in the phosphorylation of phospholipase Cγ1 (PLCγ1)(Berg et al., 2005; Reynolds et al., 2004). Phosphorylation of PLCγ1 by ITK (Beach et al., 2007) catalyzes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP2) into the secondary messenger molecules diacylglycerol (DAG) and inositol trisphosphate (IP3). DAG activates serine/threonine protein kinase C (PKCθ) (Hayashi and Altman, 2007). PKCθ phosphorylates adaptor protein CARMA1, signal transducers BCL10 and MALT1 promoting activation of transcription factor nuclear factor-κB (NF-κB) (Thome et al., 2010). IP3 binding to calcium channels triggers the release of $Ca2^{+}$ from the endoplasmic reticulum. Mobilized $Ca2^{+}$ activates the calcium-binding protein calmodulin. $Ca2^{+}$-calmodulin complex activates the serine/threonine phosphatase calcineurin which induces Il2 transcription through NFAT (nuclear factor

of activated T cells) transcription factor resulting in T cell survival (Bandyopadhyay et al., 2007; Dolmetsch et al., 1997).

2.2.2 Signal Two

CD28 is a potent co-stimulatory molecule that mediates a myriad of functions in T cells. It recruits phosphatidylinositol 3-kinase (PI3K) and promotes cell proliferation via CD28-PI3K-AKT (Pagès et al., 1994). A significant overlap is observed in the signaling activity of TCR–CD3 and CD28 and inducible T cell co-stimulator (ICOS) receptors (Smith-Garvin et al., 2009). Co-stimulation promotes induction of several downstream targets, i.e. NFAT, NF-κB, BCL-XL, mammalian target of rapamycin (mTOR) and glucose transporter type 1 (GLUT1), that prime the expression of multiple cell surface receptors and cytokines, leading to differentiation to the T helper cell subsets.

2.2.3 Signal Three

Signal three is cytokine dependent, the fate of naïve $CD4^+$ T helper cell differentiation to gain effector T cell function is determined by the cytokine milieu experienced by antigen-activated $CD4^+$ T cells. Signal three promotes the chromatin remodeling much needed for transcription of genes related to differentiation and effector functions (Curtsinger and Mescher, 2010). (Dealt separately in the following section 2.3 T helper differentiation)

2.3 T helper cell differentiation

In 1986, Mossmann and Coffman observed two different clones of $CD4^+$ T helper cells based on their specific cytokine secretion profiles upon antigenic stimulation (Mosmann et al., 1986). Their study led to classification of T helper cells into T helper type 1 cells (Th1) that secrete cytokines IFN-γ and TNF-α and T helper 2 cells (Th2) that secrete IL-4, IL-5, and IL-13 (Yamane and Paul, 2013). Subsequently, diverse subsets of T helper cells with unique functions have been identified such as Treg, Th17, Th9, Th22 and Tfh (**Figure 2**).

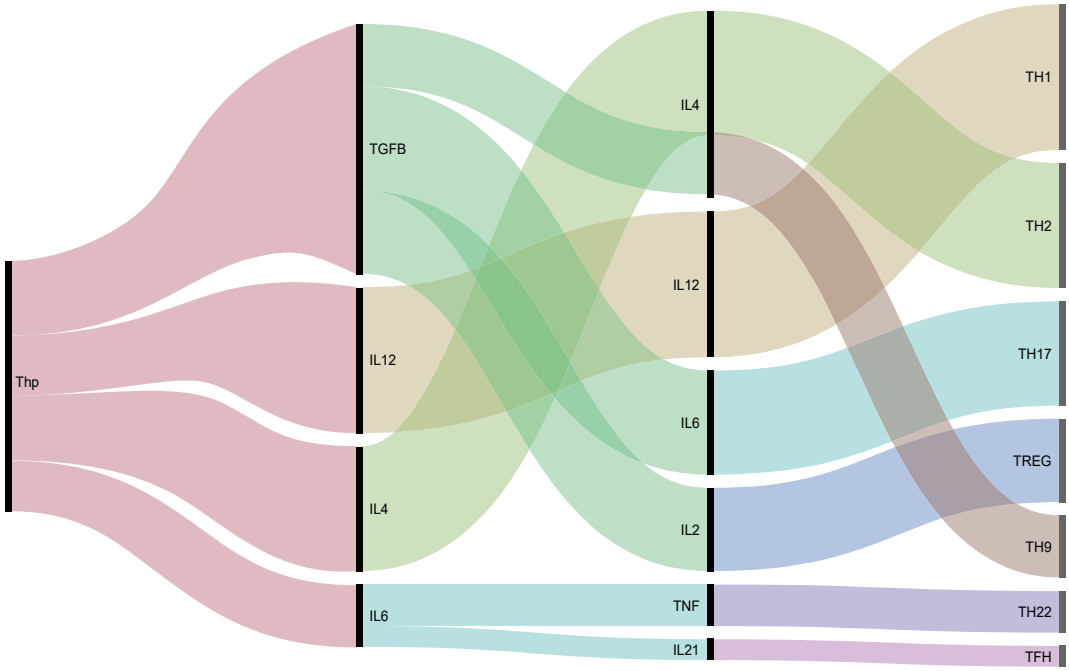


Figure 2: Path of naïve T cell differentiation to different effector cells under the influence of different cytokines. Thp: naïve T cell, TGFB: Transforming growth factor beta, IL12: Interleukin 12, IL4: Interleukin 4, IL6: Interleukin6, TNF: tumor necrosis factor alpha, IL21: Interleukin 21, TH1: T helper 1 cells, TH2: T helper 2 cells, TH17: T helper 17 cells, TREG: Regulatory T cells, TH9: T helper 9 cells, TH22: T helper 22 cells, TFH: T follicular helper cells

2.3.1 Th1 cells

Th1 cells mount the immune responses against viral and bacterial pathogens (Hsieh et al., 1993; Zhu et al., 2010). Cytokines such as IFN-γ, IFN-α and IL-12 promotes the Th1 cell lineage commitment. IL-12 phosphorylates janus kinase 2 (Jak2) and tyrosine kinase 2 (Tyk2), activates the STAT4 transcription factor that plays a pivotal role in Th1 cell commitment (Bacon et al., 1995a, 1995b; Jacobson et al., 1995). Deletion of STAT4 impairs the Th1 cell development (Thieu et al., 2008) and promotes the propensity to differentiate into Th2 cells (Kaplan et al., 1996a). Tbet, the master transcription regulator (Szabo et al., 2000) acts along with STAT4 to complete Th1 cell differentiation (Thieu et al., 2008). IL-12 receptor subunit (Il12Rβ2) is not expressed in naïve T cells, which makes them unresponsive to IL-12 signaling (Chang et al., 1999).

TCR signaling induces Tbet (Afkarian et al., 2002; Lighvani et al., 2001) and also the expression of receptor Il12Rβ2 (Szabo et al., 1997). Tbet and IL-12 are important factors that induce and maintain the production of IFN-γ (Zhu et al., 2012). IFN-γ signaling is important for Th1 development, together with STAT1 signaling IFN-γ further induces the transcription of Tbet and the IFN-γ production via a positive feed-forward loop (Afkarian et al., 2002).

2.3.2 Th2 cells

Th2 cells provide defense against helminthic infections and also contribute to allergic conditions (Zhu et al., 2010). Th2 controls antibody production of B cells by class switching to immunoglobulin G1 (IgG1) and Immunoglobulin E (IgE) (Coffman et al., 1986). IL-4 is the key cytokine that induces Th2 cell differentiation (Kopf et al., 1993; Le Gros et al., 1990; Swain et al., 1990). IL-4R is expressed in naïve CD4 T cells, IL-4 activates the transcription factor STAT6 (Kaplan et al., 1996a) and Gata3. Gata3 is the master transcription regulator of Th2 cells (Zheng and Flavell, 1997). Gata3 is also expressed in T cell precursors and plays a role in T cell development (Shah and Zuniga-Pflucker, 2014). Deletion of Gata3 modestly affects IL-4 production, however, completely abrogates production of IL-5 and IL-13 (Zhu et al., 2004). Gata3 binding sites have been observed in the promoter regions of Il5, Il13 and in enhancer regions of Il4 (Agarwal et al., 2000). TCR induced Erk/Mitogen-activated protein kinase (MAPK) elevates the expression of Gata3 (Nakayama and Yamashita, 2010). Epigenetic regulation of PcG and TrxG proteins binding to the gene locus of Gata3 governs the set of Th2 genes Il4, Il5 and Il13 (Ansel et al., 2006; Onodera et al., 2010). Deletion of STAT6 in CD4 T cells indicated that STAT6 is necessary for IL-4 induced functions in CD4 T cells including CD23 induction, MHC-class II upregulation, class switching to IgE and Th2 cell development (Kaplan et al., 1996b; Shimoda et al., 1996; Takeda et al., 1996).

2.3.3 Treg cells

Treg cells maintain the immune tolerance and homeostasis by suppressing the reactions of effector Th subsets cells towards self-molecules, allergens, and commensals. Tregs

are characterized by their secretion of soluble messengers with suppressive functions, such as TGF-β, IL-10 and adenosine. In 1995, Sakaguchi et al. reported that subpopulations of $CD4^+$ $CD25^+$ T cells are capable of suppressing autoreactive T cells *in vivo* (Sakaguchi et al., 1995). Treg cells are regulatory T cells that arise naturally (nTregs or tTregs) in thymus and in the periphery (pTregs) or *in vitro* (iTregs) from naïve T cells (Tconv). tTreg cells are selected in the thymus after the strong TCR signals. pTreg cells are generated *in vivo* and enriched in specific organs such as gut and maternal placenta. iTregs are generated *in vitro* by TCR stimulation in the presence of cytokines IL-2 (Horwitz et al., 2008) and transforming growth factor beta 1 (Chen et al., 2003). At the molecular level, Foxp3 (forkhead or winged helix family of transcription factors) has been characterized as a lineage-specifying transcription factor or master transcriptional regulator of Tregs cells (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). The mutation in the Foxp3 gene causes dysfunction of human Treg cells and results in immune dysregulation in polyendocrinopathy enteropathy X-linked syndrome (IPEX) patients (Bennett et al., 2001). Scurfy mice have a deletion in the forkhead domain of Foxp3, leading to fatal autoimmune-like disease (Khattri et al., 2003). Although Foxp3 is the master regulator of Treg cells, it does not necessarily induce all the signature genes responsible for the Treg phenotype. Foxp3 utilizes the pre-established enhancers occupied by its partners. Through binding with its cofactors, Foxp3 regulates the transcriptional repression and activation of its target genes and orchestrates the Treg cell-type response and phenotype (Samstein et al., 2012).

TGF-β converts peripheral naive $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ suppressor cells with the induction of Foxp3 (Chen et al., 2003). TGF-β triggers heterodimer formation of the proteins mothers against decapentaplegic homologs 3 and 4 (SMAD3-SMAD4) and binding of the complex to conserved noncoding sequence 1 (CNS1) in the Foxp3 gene (Xu et al., 2010). This event is important for induction of Foxp3 expression in both peripheral and *in vitro* Treg cells and helps in diverging away from naïve $CD4^+$ T cells. Single KO mice of Smad2 and Smad3 indicated the essential role of these proteins in TGF-β mediated induction of Foxp3 (Takimoto et al., 2010). Smad2 and Smad3 deleted mice had normal numbers of tTreg cells but in double KO mice of Smad2/3 the numbers of Treg cells in periphery are reduced and led to death of mice

within 3-5 weeks (Takimoto et al., 2010). The role of TGF-β signaling in the induction of tTreg development is controversial (Lu et al., 2017). TGF-β signaling may have a role in tTreg cell development, as mice without transforming growth factor-β receptor I (TβRI) produced fewer $CD4^+Foxp3^+$ thymocytes (Liu et al., 2008). In other studies, TGF-β signaling is required only for the survival of nTreg cells (Chen et al., 2001) but not for the expression of Foxp3 (Ouyang et al., 2010).

IL-2 along with cytokine TGF-β is essential for extra-thymic Treg cell differentiation. IL-2 is required for the maintenance of Treg cells (Chen et al., 2011) and unlike other cell types, Tregs express high levels of the IL-2 receptor subunit alpha (IL-2Rα, CD25). Mice deficient in common-gamma-chain (IL-2γ_c; CD132) (Burchill et al., 2007) and IL-2/IL-15 double KO mice (Burchill et al., 2007) lack Treg cells indicating that IL-2 and IL-15 signaling are critical for tTreg cell differentiation. ATRA (All-trans retinoic acid), a vitamin A metabolite, has also been shown to regulate Treg cell differentiation (Mucida et al., 2007, 2009). ATRA maintains the nTregs stability in inflammatory conditions and is critical in gut homing properties (Lu et al., 2014; Zhou et al., 2010).

2.3.4 Th17 cells

IL-17 (IL-17A/IL-17F) producing Th17 cells are a distinct and novel subset of T cells (Harrington et al., 2005; Park et al., 2005) with a role in tissue inflammation and the pathogenesis of autoimmune diseases (such as rheumatoid arthritis, inflammatory bowel diseases, psoriasis and Sjögren's syndrome). Th17 cells also contributes to clearance of extracellular pathogens including bacteria and fungi (Aujla et al., 2008; Cho et al., 2010; Ishigame et al., 2009). The discovery of Th17 cells as a novel subset started with the breakthrough observation of the p40 subunit (Oppmann et al., 2000) that is shared with IL-12 and IL-23. Studies performed in IL-23 ($p19^{-/-}$), IL-12 ($p35^{-/-}$) or both cytokines ($p40^{-/-}$) deficient mice indicated that deletion of IL-23 ($p19^{-/-}$ and $p40^{-/-}$ mice) but not IL-12 ($p35^{-/-}$) protected mice from development of EAE and collagen-induced arthritis (CIA) (Cua et al., 2003; Langrish et al., 2005). However, it was shown later that IL-23 is critical only for stabilization of the Th17 phenotype and generation of pathogenic Th17 cells, but not for differentiation of Th17 cells as the IL-23 receptor is not expressed on naïve $CD4^+$ T cells. For differentiation to Th17 cells

from naïve $CD4^+$ T cells, cytokines TGF-β (Manel et al., 2008) and IL-6 (Zhou et al., 2007) are needed with the induction of a unique master transcription factor called retinoic acid-related orphan receptor gamma t (RORC, RORγt) (Ivanov et al., 2006). The differentiation of Th17 cells is initiated after IL-6 cytokine binding to receptor complex of IL-6R and gp130 to activate STAT3 signaling that induces IL-17 production via the mediation of RORγt (Bhaumik and Basu, 2017; Lee and Kuchroo, 2015; Zhou et al., 2007). Restricted Th17 cell development (Bettelli et al., 2006) and also resistance to EAE development (Samoilova et al., 1998) have been observed in IL-6 deficient mice, indicating the importance of IL-6 in Th17 differentiation. Another transcription factor critical in the development of Th17 cells is STAT3 (Laurence et al., 2007; Mathur et al., 2007; Yang et al., 2007). T cell specific STAT3 deleted mice failed to develop colitis and also have a defect in their Th17 cell differentiation (Durant et al., 2010). Additionally, a ChIP-seq study indicated that STAT3 regulates its target genes Il17a, Il17f (Chen et al., 2006) and Il23r transcriptionally and Rorc, Batf, and Irf4 epigenetically (Durant et al., 2010). In human, mutation in the STAT3 gene causes hyper-immunoglobulin E (IgE) syndrome (HIES)(Holland et al., 2007), the loss of transcription factor STAT3 function in disease condition inhibits the development of Th17 cells leading to recurrent infections of *Candida albicans* and *Staphylococcus aureus* (Ma et al., 2008; Zhou et al., 2009). Together, STAT3 and RORC cooperate to facilitate optimal Th17 differentiation (Zhou et al., 2007).

The role of TGF-β in the differentiation of Th17 cells is controversial. A few studies have indicated the need of TGF-β as a critical cytokine for the commitment of Th17 cells development (Manel et al., 2008; Veldhoen et al., 2006; Volpe et al., 2008; Yang et al., 2008). TGF-β inhibits the differentiation of Th1 and Th2 cell *in vitro* from naïve T cells by downregulating the expression of T-bet and GATA-3 respectively (Gorelik et al., 2000, 2002). Specific deletion of TGF-β in T cells showed the highly reduced frequency of IL-17-producing cells (Gutcher et al., 2011) and an increase of IFN-γ and IL-4 production indicating the role of TGF-β in downregulating Th1 and Th2 differentiation. However, others studies indicated TGF-β is not essential for Th17 development (Acosta-Rodriguez et al., 2007; Ghoreschi et al., 2010). Th17 cells differentiated without TGF-β express Th1 cytokines such as IFN-γ and observed

greater numbers of IL-17$^+$ IFN-γ^+ cells with severe EAE disease in mice (Ghoreschi et al., 2010; Volpe et al., 2008).

2.3.5 Tfh cells

T follicular helper (Tfh) cells control germinal center (GC) responses, B-cell development, class switch and affinity maturation (Crotty, 2011). Tfh cells were identified as a novel subset on the basis of their expression of transcription factor Bcl6 (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009) and CXC chemokine receptor 5 (CXCR5)(Breitfeld et al., 2000; Kim et al., 2001; Schaerli et al., 2000). Additionally, they are characterized by their expression of PD-1, BTLA4, ICOS and SAP (SLAM-associated protein) and downregulated levels of CD127, PSGL1, and EBI2 (Epstein-Barr virus-induced G protein-coupled receptor 2). Tfh cell differentiation is a multistage process. In the early phase of Tfh cell differentiation, dendritic cells (DC) prime naive CD4$^+$ T cells in the presence of IL-6, IL-2, inducible co-stimulator (ICOS) and TCR signaling (Choi et al., 2011). This is followed by Bcl6 up-regulation and CXCR5-directed homing, whereby the Tfh cells migrate to the follicles of secondary lymphoid tissues where they interact with B cells to form ICOS and ICOS ligand (ICOSL) binding to transform into mature GC Tfh cells (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). Dysregulation of Tfh cells is linked to the development of human systemic lupus erythematosus (SLE) autoimmune disease. In SLE patients, disease levels are correlated with increased frequency of circulating Tfh cells and elevated serum auto-antibody levels in the blood (Blanco et al., 2016; Xu et al., 2015).

2.4 T helper cell plasticity

CD4 T helper cells confer immune responses by producing distinct cytokines. However, in inflammatory conditions, a CD4$^+$ T cell can exhibit the characteristics of many T cell subsets by secreting indefinite types of cytokines (DuPage and Bluestone, 2016). For example, Th17 cells convert to IFN-γ^+ producers, a feature observed in pathogenesis of autoimmune diseases (Lee et al., 2009; Shi et al., 2008). IL-17 producing Th2 cells were observed in allergic asthma patients (Wang et al., 2010). In

atopic dermatitis, T cells initially exhibited Th2 type characteristics and later become Th1 cells. In inflammatory conditions, Tregs loose Foxp3 and produce effector cytokines, such as IFN-γ, IL-2 and IL-17 (Komatsu et al., 2009, 2014; Xu et al., 2007).

2.5 T helper cell epigenetic regulation

Adaptive immune cells exhibit remarkable functional diversity and plasticity. Apart from transcriptional mediated regulation, cells utilize the epigenetic mode of regulation to attain diverse phenotypes. The major mechanisms involved in epigenetic regulation of gene expression are DNA methylation and histone modification.

2.5.1 DNA methylation

An epigenetic mark 5-methylcytosine (5mC) is a covalent modification of cytosine at 5-position established by DNA methyltransferases (DNMTs) DNMT3A, DNMT3B and DNMT1(Goll and Bestor, 2005). The cytosine methylation predominantly occurs on CG dinucleotides (CpG) forming CpG dense regions called CpG islands, localized primarily at transcriptional start sites (TSSs). DNA methylation is associated with gene repression albeit in gene bodies it is associated with transcriptional activation (Jones, 2012). The disruption of transcription-binding sites in gene promoters by CpG methylation leads to gene repression (Iguchi-Ariga and Schaffner, 1989). Conditional deletion of Dnmt1 ($CD4^+$cre) in mice decreased proliferation and increased cytokine production in naïve T cells (Lee et al., 2001). Inhibition of methylation by DNMT inhibitor 5-azacytidine induced Foxp3 expression in $CD4^+CD25^-$ cells (Kim and Leonard, 2007). T cell specific Dnmt3a deleted mice exhibited an increase in precursors of $CD8^+$ T cells and decreased $CD8^+$ T-cell effectors after viral infection (Ladle et al., 2016). Demethylation of the Ifng and Il4 loci is associated with active cytokine expression in Th1 and Th2 cells, respectively (Ansel et al., 2006). The discovery of demethylases proteins ten-eleven-translocation (TET) family of dioxygenases (TET1, TET2 and TET3) revolutionized the understanding of DNA cytosine modification (Pastor et al., 2013; Tahiliani et al., 2009). Absence of Tet2 and Tet3 in T cells resulted in uncontrolled expansion of iNKT cells leading to lethal lymphoproliferative disease (Tsagaratou et al., 2017). Tet1 and Tet2 deletion also

resulted in hyper methylation of Foxp3, decreased Treg cell differentiation and function, and immune homeostasis (Yang et al., 2015). In Th17 cells, deletion of Tet2 resulted in reduction of 5hmC and transcription factors binding and reduced cytokine expression (Ichiyama et al., 2015).

2.5.2 Histone modifications

Histone modifications accumulated during the T cell differentiation are correlated with gene expression patterns (Wei et al., 2009). Over 60 different residues are chemically modified on amino-terminal and carboxy-terminal tails of histones (Kouzarides, 2007). Histone methylation is associated with active (H3K4me3) or repressive (H3K27me3) gene expression and acetylation is associated with active transcription (Strahl and Allis, 2000). For instance, Tbx21 promoter is associated with H3K4me3 modification in Th1 cells and H3K27me3 in Th2 and Th17 cells (Wei et al., 2009). Also, DNase I hypersensitive sites were observed in the Ifng locus of in Th1 cells, but not in Th2 cells (Mukasa et al., 2010). In Th17 cells, cytokine genes Il17a and Il17f are associated with active histone marks like H3K27Ac and H3K4me3 (Akimzhanov et al., 2007).

2.6 T helper cell metabolism

Metabolic changes have been recently recognized as essential drivers in determining the fate of T helper cell differentiation. During the process of Th subset differentiation, cells undergo a massive energy demand with rapid proliferation, cytokine production, and effector functions. Immune cells possess the inherent ability to adapt their states from resting to activated in response to the external signals in order to exert immune responses (Jones and Pearce, 2017). Unstimulated thymocytes generate 96% of their ATP via oxidative phosphorylation (OXPHOS) (Guppy et al., 1993). Similarly, naïve T cells are quiescent and metabolically inactive utilize OXPHOS an efficient way of generating ATP from glucose substrate for their bioenergetic needs (Almeida et al., 2016; MacIver et al., 2013; McKinney and Smith, 2018). In response to TCR activation, activated T cells interchangeably utilize OXPHOS and aerobic glycolysis to meet bioenergetic demands. OXPHOS is fueled for T cell proliferation and survival and aerobic glycolysis to achieve the effector status. Activated T cell switch to an

inefficient aerobic glycolysis process which helps in the release of cytokines IL-2 and IFN-γ (as GAPDH inhibits translation of IFN-γ mRNA) (Chang et al., 2013; McKinney and Smith, 2018).

mTOR (mechanistic target of rapamycin) a serine/threonine kinase, plays a pivotal role in metabolic control. mTOR is associated with scaffolding protein Raptor to form mTORC1 complex and with Rictor to form a mTORC2 complex (Powell and Delgoffe, 2010). mTORC1 regulates cellular growth, proliferation, and mRNA translation (cap-dependent translation initiation). mTORC2 mediates actin cytoskeleton organization, enhances glycolytic metabolism by stimulating hexokinase 2 (HK2) and signal transduction of AGC kinase family proteins, such as Akt, SGK1 and protein kinase C (Dang, 2012; Laplante and Sabatini, 2009). mTORC1 activity is determined by measuring phosphorylation of S6 kinase (S6K1) and eukaryotic translation initiation factor 4E (eIF4E) binding proteins (4E-BPs) and mTORC2 activity by measuring phosphorylation of Akt kinase at serine 473 (Jones and Pearce, 2017; Pollizzi and Powell, 2015).

Treatment with rapamycin and genetic deletion of mTOR (targeting both mTORC1 and mTORC2) resulted in impaired Th1, Th2 and Th17 effector cell differentiation and increased Treg cell differentiation (Delgoffe et al., 2009). Specifically targeting mTORC1 (deletion of upstream protein Rheb) inhibited Th1 and Th17 cells without affecting Th2 cells (Delgoffe et al., 2011). Deletion of Raptor and Rictor showed that mTORC1 but not mTORC2 is required for differentiation of Th17 cells (Kurebayashi et al., 2012). Another study reported inactivation of mTORC1 inhibited Th2 development (Yang et al., 2013). Conversely, loss of both mTORC1 and mTORC2 in naive Th cells led to induction of Foxp3 (Delgoffe et al., 2011). Considering the possible role of mTORC1 in signaling glycolysis and mTORC2 mediating Akt-dependent glycolytic metabolism, mTORC1 and mTORC2 are dispensable in Treg differentiation. *In vitro* differentiated Treg cells showed less glycolytic metabolism and more of fatty acid oxidation (FAO) compared to effector T cells (Berod et al., 2014; Michalek et al., 2011). Treg cells also express high levels of carnitine palmitoyltransferase (Cpt1a), rate-limiting enzyme of FAO, suggesting Tregs depend on FAO for metabolic demands. Additionally, disruption of GLUT1 (glucose transporter) restricted Th1, Th2, and Th17 but not Treg cells (Macintyre et al., 2014)

suggesting Treg cells does not rely on glycolysis. In nTregs and iTregs, there is higher activity of AMP-activated protein kinase (AMPK) (Michalek et al., 2011). Treating naïve T cells with metformin (an AMPK activator) favors Treg differentiation by increasing lipid oxidation activity and impairs the Th1/Th17 differentiation (Kang et al., 2013; Zhao et al., 2015).

2.7 Biology of Estrogen receptor alpha

$CD4^+$ T helper cells are arguably the most important cells in adaptive immunity with critical role in autoimmunity. Women are more prone to autoimmune diseases compared to men. The sex hormone estrogen secreted in females might play an important role in controlling the autoimmune diseases. Estrogen receptor alpha (ERα) is a receptor for estrogen hormone (17β-estradiol, E2). The E2 regulates the transcription of its target genes through ERα by binding to high affinity estrogen-responsive elements (ERE) on DNA (Mangelsdorf et al., 1995). ERα binds to the genes responsible for cell proliferation with a critical role during mammary gland development and pregnancy.

2.7.1 Gene and protein structure

ERα is encoded by the Esr1 gene located on human chromosome 6q25 with a gene size of about 140 kilobases and consisting of eight coding exons interrupted by seven introns (Green et al., 1986; Ponglikitmongkol et al., 1988). In mouse, Esr1 is mapped on chromosome 10 and the full-length ERα has a molecular weight of 66 kDa and consists of 595 amino acids (Green et al., 1986; Justice et al., 1990). ERα is composed of five distinct domains: The N-terminal domain contains the activation function (AF-1) region, which interacts with co-regulators during transcription even in the absence of ligand. The DNA-binding domain (DBD) binds to estrogen response elements (EREs) in DNA and enables gene expression events. A structural hinge domain (D) contributes to nuclear localization signaling and post-translational modification of ERα. The AF2 domain contains a ligand-binding domain (LBD; E/F domain). The AF2 domain is the transactivation domain facilitates interaction with co-regulators in the presence of ligand (Berry et al., 1990; Dahlman-Wright et al., 2006).

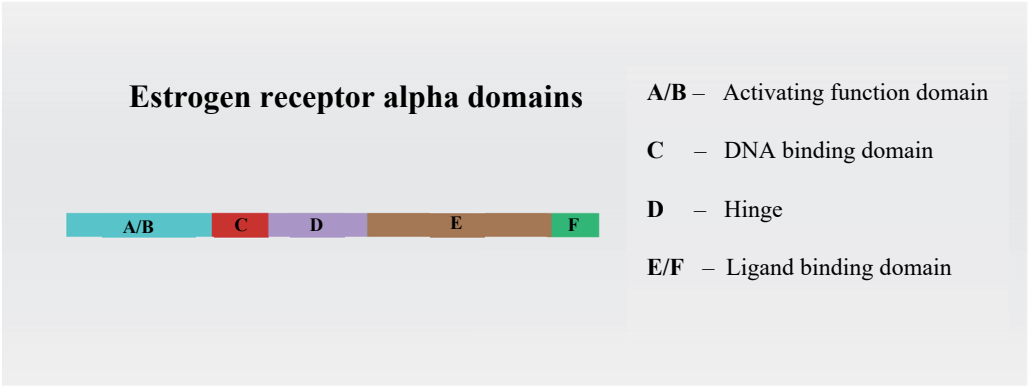


Figure 3: Figure showing 5 distinct domains of human ERα proteins along with their molecular functions. Adapted from Jia et al., 2015.

2.7.2 Isoforms of ERα

Functionally characterized protein isoforms of ERα are of 66 kDa, 46 kDa and 36 kDa in size. Out of the five domains in the full length isoform (ERα66) , N-terminal domain (AF1) and c-terminal domain (AF2) are responsible for activation function (responsible for recruitment of co-activator) in MCF7 cells (Flouriot et al., 2000) (Figure 3). Truncated isoform ERα 46 is devoid of AF-1 domain hence not capable of initiating estradiol-mediated cell proliferation. Short isoform ERα 36 lacks both AF1 and AF2 domains and does show any transcriptional activity but forms heterodimers with full-length ERα protein (McInerney and Katzenellenbogen, 1996; Nilsson et al., 2001; Ribeiro et al., 1995).

2.7.3 Estrogen and ERα in immune responses

ERα and ERβ are expressed in thymocytes, T cells and B cells, indicating that estrogen can exert its actions on immune cells (Haruki et al., 1983; Shim et al., 2006). Accordingly, estrogen has been shown to alter both the innate and adaptive immune system (Straub, 2007). In macrophages, estrogens regulate phagocytic activity, induction of cytokines, chemotaxis, nitric oxide and iNOS (Dai et al., 2008; Hsieh et al., 2009; Karpuzoglu et al., 2009). Estrogens promote differentiation of immature dendritic cells to mature DCs by influencing the expression of IL-6, IL-10, CXCL8, and CCL2 (Bachy et al., 2008; Liu et al., 2002). $CD4^+$ T cells express higher levels of

ERα than ERβ; B cells express higher levels of ERβ than ERα. $CD8^+$ cells in comparison to $CD4^+$ and B cells express lower but equivalent levels of ERα and ERβ (Phiel et al., 2005).

2.8 Estrogen and autoimmune inflammation

Autoimmune diseases have higher incidence in females. 80% of autoimmune patients are women (Rubtsova et al., 2015). Estrogen exerts a biphasic effect in SLE and MS autoimmune diseases. It has a deleterious role in SLE and beneficial effect in MS (Ascenzi et al., 2006). Estrogen hormones also have impact in pathogenesis of IBD (Verdú et al., 2002) as discussed below.

2.8.1 IBD

Inflammatory bowel disease (IBD) is a chronic inflammation classified as Crohn disease (CD) and ulcerative colitis (UC). The symptoms of disease includes bloody diarrhea, fatigue and weight loss (Baumgart and Sandborn, 2007). Developmental risk of IBD in women has been linked with use of oral contraceptives (Godet et al., 1995; Khalili et al., 2013). During pregnancy, women are protected from IBD disease flare-ups (Riis et al., 2006). Reduced expression of estrogen receptor β in peripheral blood T lymphocytes from CD/UC patients has been reported, suggesting that the ER profile is influenced in IBD patients (Pierdominici et al., 2015).

2.8.2 SLE

SLE is a complex autoimmune disease characterized by defective regulation of B- and T-lymphocytes causing immune cell hyperactivity with the production of antinuclear auto-antibodies (Deng et al., 2013). The predominance of SLE is observed 10 times more in women than men (Weckerle and Niewold, 2011). Estrogen levels in serum are much higher in SLE patients than in healthy subjects (Folomeev et al., 1992). Estrogen enhanced anti-double-stranded DNA antibody, IgG and IgM production *in vitro* cultured human PBMCs (Kanda and Tamaki, 1999). Estrogen also promoted systemic inflammation and induction of B cell activating factor and IFN

signature genes in an SLE mouse model (Venkatesh et al., 2011). Increased ERα and decreased ERβ mRNA expression has been observed in PBMCs of SLE patients (Inui et al., 2007). The association of an ERα genetic polymorphism has also been reported in patients with SLE (Drehmer et al., 2017; Johansson et al., 2005; Lee et al., 2004). In an SLE model, estrogen induced cytokine modulation was mediated by ERα and its disruption alleviated the disease (Bynoté et al., 2008).

2.8.3 MS

Multiple sclerosis is an autoimmune, neurodegenerative disease characterized by demyelination of axons causing axonal loss, the presence of infiltration of myelin antigen reactive $CD4^+$ T cells in CNS (Frohman et al., 2006; Pettinelli and McFarlin, 1981). Estrogen hormone has been reported to have ERα-mediated anti-inflammatory and neuroprotective action in MS. The hormone reduces the levels of pro-inflammatory mediators (such as IFN-γ, IL-17, TNFα, MCP-1, and iNOS) and extent of inflammation and demyelination in disease conditions (Lélu et al., 2011; Offner and Polanczyk, 2006; Polanczyk et al., 2003). Experimental autoimmune encephalomyelitis (EAE) is the most widely used rodent model of MS. In EAE model, ERα-ligand treatment alleviated EAE *in vivo* by acting on lymphocytes and astrocytes (Spence et al., 2013). Estrogen also affects $CD4^+$ T cells expansion, increases T cell apoptosis and proportion of Treg and $CD4^+CD8^-$ suppressor T cells (Pettersson et al., 2004; Xiao et al., 2004). In pregnant EAE mice, decreased EAE and reduced levels of IL-17 and TNF-α are produced in comparison to non-pregnant controls (Gatson et al., 2011).

2.9 The mechanism of estrogen action

2.9.1 Genomic

Upon activation with estrogen hormone, ERα dissociates from its heat shock proteins complex, forms homo or hetero-dimers and translocate into the nucleus (Ylikomi et al., 1998). The Estrogen receptor and hormone complex recruits co-activators, co-repressors and chromatin remodeling factors, which influence transcription by either activating or repressing target genes (Bocquel et al., 1989; Meyer et al., 1989). In

genomic dependent estrogen action, ERα binds directly to ERE palindromic consensus DNA sequences (Klinge, 2001). ERα shows high specificity binding to 13-base-pair inverted-repeat DNA sequence (GGTCAnnnTGACC) present in promoter/enhancer regions of target genes (Klein-Hitpass et al., 1988; Mader et al., 1989).

2.9.2 Non- Genomic

In cells, estrogen modulates cell signaling pathways very rapidly through estrogen receptors located at the plasma membrane and cytoplasm called G-protein-coupled-estrogen receptor 1(GPER)(Revankar et al., 2005). Activated GPERs induce the production of cAMP and intracellular calcium mobilization and activate PI3K signaling (Revankar et al., 2005). The rapid mechanism was observed initially in ovariectomized rats administered with E2 showed the increased levels of uterine cAMP levels within 15 seconds (Szego and Davis, 1967).

2.9.3 Ligand-independent

This mode of cell signaling takes place in the absence of estrogen ligand. Signaling molecules (e.g. PKA, PKC, and MAPK) stimulate ER-dependent transcription by phosphorylating ERα receptor. Growth factors such as insulin-like growth factor-1(IGF-1) and epidermal growth factor (EGF) activate mitogen-activated protein kinase (MAPK) dependent signaling. MAPK subsequently phosphorylates ser118 in the AF1 domain of estrogen receptor (Kato et al., 1995). Alternatively, ER activation in the absence of ligands is carried out via cAMP signaling. cAMP-activated protein kinase A (PKA) phosphorylates ERα then dimerize and binds to their EREs to regulate gene transcription check (Moss and Gerton, 2001).

2.9.4 ERE independent

In this mode of action, ER acts as co-regulator rather than transcription factor. Upon activation of ER with estrogen, ER does not bind to ERE sequence. Instead, it interacts with other DNA- binding transcription factors such as activating protein (AP-1/c-Jun), c-Fos, ATF-2, nuclear factor-kB (NF-kB), Stimulating protein-1 (Sp1) and Sp3 to

initiate gene transcription (Björnström and Sjöberg, 2005). This mechanism is also referred as transcription factor cross-talk. After stimulation with E2, Sp-1 interacts with ERα and initiates the transcription of endothelial nitric oxide synthase (eNOS), c-fos, cyclin D1, low-density lipoprotein LDL) receptor and the retinoic acid receptor-1 genes (Marino et al., 2006). In the presence of E2, ERα interacts with a c-rel subunit of NF-kB complex to inhibit expression of IL-6 cytokine in HeLa, MCF7 and Saos2 cell lines (Galien and Garcia, 1997; Ray et al., 1994).

2.10 Biology of vimentin

Vimentin is an abundant cytoskeletal protein that belongs to the group of type III intermediate filaments (**Figure 4**). Vimentin is induced in mesenchymal cells and it acts as a marker to determine epithelial-mesenchymal transition (EMT). It provides the mechanical strength, maintaining cell shape by stabilizing cytoskeletal interactions, cell attachment, migration and signaling during embryogenesis and metastasis (Eriksson et al., 2009; Lowery et al., 2015).

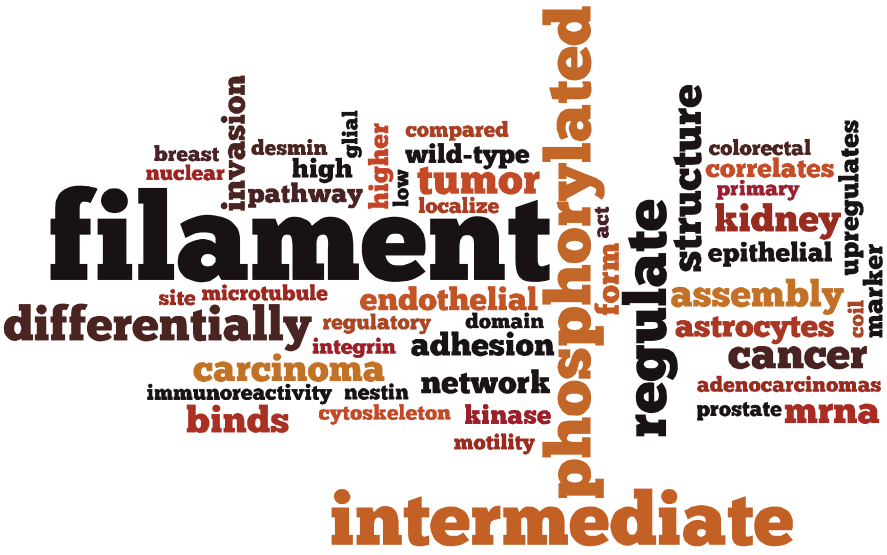


Figure 4: Illustration indicating the biological theme of gene vimentin. Pubmed (GeneRIF annotation) and the Genes2WordCloud tool were used to obtain the frequency of term (Baroukh et al., 2011). Bigger the font size of term, more it is related to vimentin gene.

In osteoblast cell lines, TGF-β cytokine was observed to induce the vimentin expression via PI3K-Akt-mTOR signaling but not through Smad signaling (Lian et al., 2012). Addition of Akt (Wortmannin) inhibitor and mTOR (Rapamycin) inhibitor, but not Smad (SB505124) inhibitor, abolished the TGF-β induced vimentin protein expression.

Vimentin acts as a scaffold for signal transduction events, it aids in the formation of signaling complexes with signaling molecules and adaptor proteins (Eriksson et al., 2009). 14-3-3 proteins belong to family of a phospho-binding proteins with critical role in signaling of many processes, such as apoptosis, cell cycle progression and autophagy (Pennington et al., 2018). Phosphorylated vimentin has been observed to interact with 14-3-3 protein, the resultant complex causes Raf-1 release leading the decrease in Raf-1 kinase activity consequently influencing the intracellular signaling (Tzivion et al., 2000).

In triple-negative breast carcinomas Slug, ERK, and vimentin were observed to be co-expressed, in a cancer related model it was observed that the vimentin-ERK-Slug axis promotes EMT induction (Virtakoivu et al., 2015). Vimentin also facilitates fibroblast proliferation and keratinocyte differentiation. Impaired wound healing in $Vim^{-/-}$ mice was reported with slow re-epithelialization and limited keratinization (Cheng et al., 2016). During inflammatory conditions, vimentin influences the transmigration ability of lymphocytes *in vivo* by affecting integrin and ICAM-1 levels (Nieminen et al., 2006). In acute lung injury (ALI) and pulmonary fibrosis settings, vimentin is essential for assembly and activation of the NLRP3 inflammasome (dos Santos et al., 2015). Vimentin interacts with NLRP3 inflammasome and promotes the IL-1β maturation. A recent study reported that Vimentin KO mice challenged with DSS (Dextran sodium sulphate model) showed reduced gut inflammation, decreased bacterial extravasation compared to WT mice (Mor-Vaknin et al., 2013). *In vitro* conditions, Vim deficient phagocytes showed the increased bacteria-killing capacity by producing the elevated levels of reactive oxygen species (ROS) and nitric oxides (Mor-Vaknin et al., 2013). However, in the reported study, it was not clear how vimentin influences functional activation of NADPH oxidase complex (Mor-Vaknin et al., 2013).

2.10.1 Factors regulating expression of vimentin

The vimentin promoter region harbors binding sites for TATA box, Activator-protein binding, NF-κB binding, eight GC boxes and a Smad binding element (Kidd et al., 2014; Satelli and Li, 2011). Hypoxia-inducible factor-1 (HIF-1) has been reported to regulate the vimentin gene expression in HCT116 human colon carcinoma cells (Krishnamachary et al., 2003). Vimentin expression correlates with invasiveness of tumor (Hendrix et al., 1996). The regulation of vimentin transcription by HIF-1 might be important in promoting EMT (Kidd et al., 2014). IL-6 induces vimentin expression during the terminal differentiation of M1 myeloid leukemia cells (Tsuru et al., 1990). Anti-inflammatory cytokines such as IL-10 (Mor-Vaknin et al., 2003) and IL-4 (Hornbeck et al., 1993) downregulate the vimentin expression in activated human macrophages and in murine T cells, respectively. Conversely, pro-inflammatory cytokines such as TNF-α promote the secretion of extra cellular vimentin in activated human macrophages (Mor-Vaknin et al., 2003). However, cytokine TGF-β induces the vimentin mRNA expression during EMT and cancer progression (Kokkinos et al., 2007; Steinert and Roop, 1988).

2.11 Established mouse models in the laboratory

2.11.1 Adoptive transfer colitis

In this model, naïve $CD4^+$ T cells ($CD4^+$ $CD45RB^{high}$ T cells) isolated from donor mice are transferred to lymphopenic $Rag^{-/-}$ or SCID mice, which lack T cells, to develop wasting disease and colonic inflammation (Powrie et al., 1993, 1994). Chronic colitis is induced 5 to 10 weeks after injection because of disruption of T cell homeostasis (Ostanin et al., 2009). It is assumed that the injected T cells migrate to the gut and become exposed to antigens and commensal microflora (Elson et al., 2005). Affected colons of mice show inflammation with epithelial cell hyperplasia, infiltration of polymorphonuclear leukocyte (PMN) and mononuclear leukocytes and crypt abscesses. Injected Naïve CD4 T cells differentiate into Tbet or Rorγt positive Th1 or Th17 cells (Mikami et al., 2010; Powrie et al., 1994). This model is a valuable tool to study the contribution of T cell populations, characterization of cytokines and adhesion molecules in the pathogenesis of IBD.

2.11.2 DSS colitis

Dextran sodium sulfate (DSS), a polyanionic derivative of dextran, is administered in drinking water to cause acute or chronic intestinal inflammation (Cooper et al., 1993; Okayasu et al., 1990). The mechanism of induction of intestinal inflammation by DSS is not clear but it is likely that DSS causes epithelial cell injury allowing luminal antigens and enteric bacteria to penetrate to the inner mucus layer triggering the inflammatory responses. The colitis is characterized by erosion, loss of crypts and infiltration of granulocytes. This model is useful in studying the contribution of the innate immune mechanism to the development of mucosal inflammation (Dieleman et al., 1994; Krieglstein et al., 2002). The cells involved in innate immune responses such as macrophages act as a source of pro-inflammatory cytokines that regulate epithelial barrier function and neutrophils infiltration contributing to tissue damage. T cell responses aggravate this response. In acute colitis -Th1 responses, and in repeated cycles of chronic DSS colitis-Th1/Th2 responses are observed. Carcinogen azoxymethane (AOM) carcinogen is administered along with DSS to induce colitis-associated tumors (Wirtz et al., 2007). Tumor development has been reported to be caused because of the presence of T cells and pro-inflammatory cytokine such as IL-6 (Kiesler et al., 2015).

2.12 Proteome characterization studies on T cells

2.12.1 Proteomic technology

The aim of proteomics is the determination of all the expressed proteins, rather than focusing on single molecule or pathway, to attain a holistic view of cellular cross talk. The advent of recent developments in technologies and bioinformatic approaches has enabled a better understanding of biological complexity. Particularly in proteomics, increased sensitivity and resolution of mass spectrometry (MS) instruments has enabled in-depth system-wide characterization of proteins. Proteomic technology can be used to study protein abundance levels (differential protein expression), protein isoforms, post-translational modifications, protein-protein interactions, sub cellular localization, and protein turnover rate (protein synthesis and degradation) (Aebersold and Mann, 2016; Breker and Schuldiner, 2014; Larance and Lamond, 2015).

2.12.2 High dimensional detection of proteins at single-cell level

Mass cytometry (CyTOF) is an emerging platform for single-cell proteomics. It is a fusion of two technologies, flow cytometry and mass spectrometry (Inductively Coupled Plasma ICP-MS) enabling measurement of over 40 parameters simultaneously on individual cells. Similar to flow cytometry, cells are labeled with antibodies but conjugated with stable heavy metal isotopes rather than fluorophores. Single-cell suspension of sample is introduced into nebulizer to form single cell droplets. In the first step of mass cytometer, cells passes through spray chamber with an approximate temperature of 200˚C causing vaporization of water around the cells. Cells are then heated that breaks covalent bonds resulting in a cloud that passes through quadrupole. The metal reporter ions are separated based on mass-to-charge ratio in time-of-flight chamber and are directed to detector.

CyTOF platform facilitates understanding of cell phenotypes and signaling pathways at single-cell resolution (Spitzer and Nolan, 2016). The applications of CyTOF include: Multidimensional analysis of mass cytometry data was first employed to identify distinct cell phenotypes in the human hematopoietic system by examining 35 different parameters (Bendall et al., 2011). The classification of tumors and prognosis of acute myeloid leukemia (AML) was performed by assessing single cell composition of AML tumors using CyTOF platform (Amir et al., 2013).

2.12.3 Strategies followed in proteomics

The strategies applied in proteomic studies mostly use either bottom-up or top-down methods (Aebersold and Mann, 2016; Breker and Schuldiner, 2014; Larance and Lamond, 2015). In a bottom-up work flow, complex protein mixtures are digested with a proteolytic reagent (Trypsin, Lys-C) and the resulting peptides are separated on high–performance liquid chromatography (HPLC) coupled to the mass spectrometer (Hosp and Mann, 2017). In the top-down method, intact proteins are subject to mass spectrometer analysis without digesting them into peptides (Toby et al., 2016). This facilitates the studying all the post-translational modifications that occur on the same molecule and determination of the protein isoform (Choudhary and Mann, 2010). In all of these methods LC is coupled to electrospray ionization (ESI), the effluent from

LC is nebulized and ionized to form charged particles. Peptide ions are separated according to mass-to-charge ratio. The charged particles under high vacuum are fragmented through a series of mass analyzers (quadrupole). The resulting product ion MS/MS (MS^2) spectra are searched against a sequence database to identify and quantify specific peptides and deduce protein in the sample (Savaryn et al., 2016).

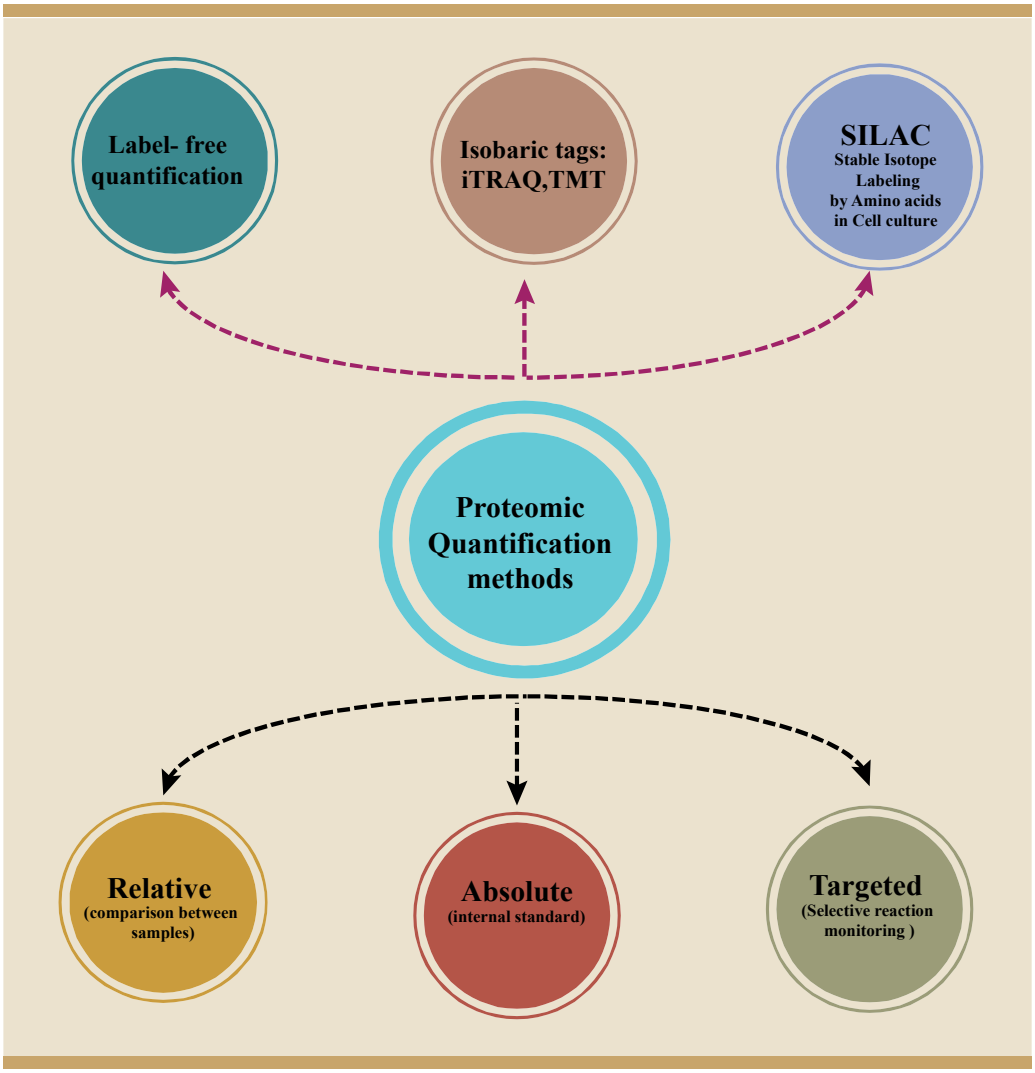


Figure 5: Illustration depicting various strategies and approaches followed in proteomic quantification methods. The arrows in burgundy color point towards the strategies that are used in sample preparation and the black color arrows point towards the quantification methods.

Bottom-up proteomics is further sub-classified into following approaches; Data-dependent acquisition (DDA), Data independent acquisition (DIA) and Targeted proteomics (Hu et al., 2016). In DDA method, data are obtained in which a fixed number of precursor ions m/z values from MS^1 (full scan) are selected and subjected to the second stage of fragmentation (at MS^2 level). In DIA method, all precursor ions in defined m/z range are fragmented and analyzed in the second stage of MS/MS analysis. This method eliminates missing value problem (certain peptides cannot be identified/quantified in samples from MS analysis) that is common in DDA. Targeted proteomics is also called as selected reaction monitoring (SRM)/multiple reaction monitoring (MRM), in which a subset of known peptides of interest are selectively isolated and then fragmented (Picotti and Aebersold, 2012). The SRM approach allows selective and sensitive quantitation (Shi et al., 2016). To attain precise quantification of peptides, chemical labeling of proteins can be carried out by approaches such as stable isotope labeling by amino acids in cell culture (SILAC) and isobaric mass tags (TMT, iTRAQ)(Rauniyar and Yates, 2014). However, label-free quantitation (LFQ) is a rapid and low-cost quantitative proteomic approach (Ahmad and Lamond, 2014; Zhang et al., 2013). With this approach, the samples are not labeled like in other methods where stable isotopes are used to chemically label the proteins. Relative protein quantification is achieved in computational framework by matching the MS/MS spectra across the samples that are measured separately, for example, using MaxQuant software. The simplified pipeline of MaxQuant LFQ includes feature detection and quantification in MS spectra (mass and intensity of the peptide peaks), first and main search, matching between runs (retention time alignment/normalization) and protein identification/ quantification (Tyanova et al., 2016).

2.12.4 Application of proteomics in T cells

T cells form critical component of the adaptive immune system. They show diverse functions from secreting cytokines to expressing surface receptors. Many different genomic technologies have been used to study T cells (Elo et al., 2010; Heng et al., 2008; Wei et al., 2009). With the recent inclusion of new mass analyzer (Orbitrap), the mass spectrometry method enabled in-depth assessment of protein profiles in T cells (**Table 1**).

Table 1: Applications of proteomics analysis of T cells

Authors	Methods	Annotation
Thiede et al., 2000	Electrophoretic separation (2DE) gels and peptide mass fingerprinting	Identified 67 proteins in Jurkat human T cell line.
Nyman et al., 2001	2-DE,MALDI-TOF, autoradiography and Silver staining	Identified 91 proteins in human CD4+ T cells.
Vuadens et al., 2002	2-DE (IEF-SDS), MS	Compared $CD19^+$ B, $CD8^+$ T, and $CD4^+$ T lymphocytes and observed the expression of vimentin, desmin, tubulin and cytokeratin in $CD4^+$ and $CD8^+$ cells but not in $CD19^+$ cells.
Rautajoki et al., 2003	2-DE, MS/MS	14 differentially expressed proteins (IFI53, VMA4, PSMA4, IFP35, PSME1, H2A, ISG15, PHB, ACT) examined from in *vitro* cultured human Th1 and Th2 cells.
Stentz and Kitabchi, 2004	proteome (using SELDI-TOF) and transcriptome (Affymetrix microarray gene chips)	Increase in receptors for insulin, IGF-1, and IL-2 receptors in activated vs non-activated human $CD4^+$ and $CD8^+$ T cells.
Rosengren et al., 2005	2-DE, MS	Identified expression changes in MIF, Pdcd4, PAK2 and Cdc42 proteins in human T helper cells treated with and without interleukin-12.
Filén et al., 2009	isotope-coded affinity tag labeling in combination with chromatographic techniques and tandem mass spectrometry	Identified 147 IL-12 regulated proteins in the microsomal fraction of Th cells.
Loyet et al., 2005	SILAC MS	Studied 38 membrane proteins on Th1 and Th2 cells and compared with microarray data.
Moulder et al., 2010	stable isotope labeling with 4-plex iTRAQ (isobaric tags for relative and absolute quantification) and LC-MS/MS	Detected 800 proteins in T cells stimulated with and without IL-4.
Kim et al., 2014	LC-MS/MS	Investigated 30 different human tissues (including 7 fetal tissues and 6 hematopoietic cell types).
Wilhelm et al., 2014	LC-MS/MS	Measured proteome in 60 human tissues, 13 body fluids, 147 cell lines and identified 18,097 proteins in the human proteome.
Hukelmann et al., 2016	LC-MS/MS	Identified 6,800 protein groups from P14 TCR transgenic $CD8^+$ cytotoxic T lymphocytes.
Aalderen et al., 2017	LC-MS/MS	7 distinct subsets of circulating CD8+ T cells from healthy adults.
Tan et al., 2017	LC-MS/MS	Integrative analysis of proteomics and phosphoproteomics to profile early TCR signaling events.
Rieckmann et al., 2017	LC-MS/MS	28 different types of human immune cells and established a social immune cell network.

3 AIMS OF THE STUDY

The overall goals of the work presented in this PhD thesis were to identify and characterize the novel molecules that regulate the differentiation of Th17 and iTreg cells. The emphasis was to first characterize the molecular mechanisms of ERα in regulation of T cells and autoimmunity with high-throughput technology such as RNA sequencing. The study also utilized the system-wide approaches to deepen the understanding of T helper differentiation.

Specifically, the aims were:

1. To study the role of estrogen receptor alpha in T cells mediated autoimmunity and identify its role in the differentiation of T helper cells. (I)
2. To profile the proteome and integrate with transcriptome of murine Th17 and iTreg cells.(II)
3. To characterize the role of vimentin in iTreg differentiation. (II)

4 MATERIALS AND METHODS

4.1 Mice and ethical considerations (I, II)

ERα$^{fl/fl}$ mice (ERα exon 3 is flanked by loxP sites) have been previously reported (Dupont 2000) and were crossed with CD4-cre mice (Taconic, Hudson, NY) to obtain T cell-specific ERα knockout mice. Rag1$^{-/-}$ mice (strain number 002216) were purchased from the Jackson Laboratory. Only female mice were used in the colitis experiments. For cell culture experiments six to sixteen week old sex-matched mice were used. For the proteomics study, eight to ten week old C57BL/6 mice were used. All the animals were housed in Central Animal Laboratory at University of Turku or the Central Animal Facility of the College of Veterinary Medicine at the University of Georgia (UGA). The mice were fed *ad libitum* and provided with 12/12 hour light and dark cycles. All the experiments were performed in accordance with the guidelines of Finnish Animal Ethics Committee and UGA Institutional Animal Care and Use Committee.

4.2 Flow cytometry (I, II)

To detect the surface expression of CD69 and CD101 (both antibodies are from BD Biosciences, San Diego, CA), *in vitro* cultured Th0, iTreg and Th17 cells were re-suspended in 2% FBS in PBS and incubated on ice for 30 min. Intracellular staining for Foxp3, Bcl2 and IL-17 (all antibodies are from BD Biosciences) was carried out using a Foxp3 staining kit (eBioscience, San Diego, CA) according to the manufacturer's instructions. Briefly, cells were washed in PBS twice and re-suspended in fix/perm buffer for 40 min. Fluorochrome-conjugated antibodies were diluted 1 to 100 and permeabilized on ice for 1 hour. To perform IL-17 cytokine detection, cells were stimulated with PMA and ionomycin for 4 hours followed by 2 hours incubation with brefeldin A in the 37˚C incubator. Cell population measurements were acquired on LSR II flow cytometer (BD Biosciences) and analyzed using Flowjo software (TreeStar).

4.3 Adoptive T cell transfer colitis (I)

Mice were sacrificed by CO_2 asphyxiation. Spleens were separated from ERα$^{fl/fl}$ and CD4-cre ERα$^{fl/fl}$ mice. Single cell suspensions were prepared by pressing them against 70μm cell strainers. Erythrocytes lysis was done by incubating suspensions in ACK lysis for 3-5 mins. Enrichment of CD4$^+$ T cells was performed from single cell suspensions with CD4$^+$ T cell isolation kit (Mitenyi Biotec, Bergisch Gladbach, Germany). Naïve T cells (CD4$^+$CD45RB^{hi}CD25$^-$) were purified from the pre-enriched CD4$^+$ T cell fraction by FACS sorting. Colitis was induced into 6- to 7- week old female Rag1$^{-/-}$ mice by injecting FACS sorted naïve CD4$^+$ T cells intravenously. The weight of the mice was monitored every week after injection.

4.4 CD4$^+$ T cell isolation (I, II)

For *in vitro* T cell activation, naïve CD4$^+$ T cells were isolated with a Miltenyi MACS CD4$^+$CD62L$^+$ T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. Briefly, the single cell suspension was incubated with a non-CD4$^+$ T cell biotin-antibody cocktail and anti-biotin microbeads. This step allows the depletion of non-CD4$^+$ T cells by separation over a MACS column to enrich CD4$^+$ T cells in the flow through. The cells from flow through were further labeled with CD62L (L-selectin) microbeads and separated by positive selection in the presence of magnetic field to obtain naïve CD4$^+$ T cells.

4.5 Culture media and conditions (I, II)

Cell cultures in the ERα study (I) were done with phenol-red free IMDM medium supplemented with 10% charcoal stripped fetal calf serum, 100 IU/mL penicillin, 0.1 mg/mL streptomycin (Sigma, St Louis, MO), 2 mM glutamine, 2.5 μM β-mercaptoethanol. ERα$^{fl/fl}$ and CD4-creERα$^{fl/fl}$ cells were cultured with anti-CD3 (1 μg/mL, BD PharMingen, San Diego, CA) and anti-CD28 (1 μg/mL, eBioscience, San Diego, CA). For *in vivo* proliferation assays, the mice used were CD4-creERα$^{fl/fl}$ mice and CD45.1$^+$ C57BL6/J congenic mice. T cells were isolated using a Pan T Cell Isolation kit (STEMCELL Technologies). Naïve CD4 T cells (CD4$^+$CD62L^{hi}CD44^{low}CD25$^-$) were further purified by FACS sorting with antibodies

CD4, CD62L, CD25, and CD44. WT ($CD45.1^+$C57BL6/J) and $CD45.2^+$CD4-cre$ER\alpha^{fl/fl}$ naïve T cells were mixed in a 1:1 proportion for CFSE labeling. One million cells were injected intravenously into $Rag1^{-/-}$ recipient mice. T cells isolated from the spleens and MLNs were analyzed for CD45.1, CD45.2, and CFSE dilution on day 10 or 14 after the mice were euthanized.

Naïve $CD4^+$ T cells were induced to the iTreg phenotype (I) by culturing in the presence of plate-bound anti-CD3 and anti-CD28 (both at 1 μg/mL). Coating of the plate was carried out for 1-3 hours at 37˚C. A cytokine cocktail containing recombinant mouse IL-2 (10 ng/ml, R&D Systems, Minneapolis, MN) and recombinant human TGF-β1 (10ng/ml) was used along with complete media for polarization.

Cell cultures for the proteomics study (II) were carried out with IMDM medium supplemented with 10% fetal calf serum, 100 IU/mL penicillin, 0.1 mg/mL streptomycin (Sigma, St Louis, MO), 2 mM glutamine and 2.5 μM β-mercaptoethanol. For iTreg polarization, naïve CD4 T cells were cultured with IL-2 (10ng/ml, R&D system, Minneapolis, MN) and recombinant human TGF-β1 (20ng/ml) for 7 days followed by re-stimulation with anti-CD3/CD28 and fresh media containing cytokines for another 3 days. For Th17 polarization, cells were cultured for three days in the presence of cytokines IL-6 (30ng/ml, PeproTech, UK), TGF-β1 (5ng/ml) and neutralizing antibodies anti-IL-4 and anti-IFNγ (both at 10 μg/mL, BD PharMingen, San Diego, CA).

4.6 *In vitro* T cell proliferation assay (I)

$CD4^+$ T cells from $ER\alpha^{fl/fl}$ and CD4-Cre $ER\alpha^{fl/fl}$ mice were isolated and labeled with the CellTrace Violet Cell Proliferation Kit (Invitrogen, Waltham, MA). Cells were incubated with 2.5 μM of dye for 20 min at 37°C for labeling. The cells were then washed with phenol-red free IMDM medium (supplemented with 10% charcoal stripped fetal calf serum, 2 mM glutamine). Cells were cultured till 4 and 5 days in an activated condition in the presence of anti-CD3 (1 μg/mL, BD PharMingen, San Diego, CA) and anti-CD28 (1 μg/mL, eBioscience, San Diego, CA). Flow cytometry detection of CTV labelled cells was carried out on an LSRII flow cytometer (BD Biosciences), and data were analyzed with FlowJo software (V.10, Tree star).

4.7 Annexin-PI staining (I)

To determine the proportion of apoptotic and pro-apoptotic $CD4^+$ T cells were stained with a combination of Annexin V-FITC and Propidium iodide (PI) followed by flow cytometry. Naïve $CD4^+$ T cells from $ER\alpha^{fl/fl}$ and CD4-cre $ER\alpha^{fl/fl}$ were activated with anti-CD3 (1 µg/mL, BD PharMingen, San Diego, CA) and anti-CD28 (1 µg/mL, eBioscience, San Diego, CA) for 3 days. The staining buffer was prepared with equal volumes of FACS buffer (2% FCS in PBS) and binding buffer (5 mM Hepes, 70 mM NaCl and 2.5 mM $CaCl_2$, pH-7.4). After washing, cells were re-suspended in staining buffer containing Annexin V-FITC (561012, BD) and PI (P4864, Sigma) and incubated in darkness at room temperature for 20 min.

4.8 Seahorse extracellular flux analysis (II)

Oxygen consumption rate (OCR) and Extracellular acidification rate (ECAR) measurements were performed with a Seahorse XFp analyzer (Agilent Technologies). Naïve $CD4^+$ T cells from $ER\alpha^{fl/fl}$ and CD4-cre $ER\alpha^{fl/fl}$ were activated with anti-CD3 (1 µg/mL, BD PharMingen, San Diego, CA) and anti-CD28 (1 µg/mL, eBioscience, San Diego, CA) and cultured for 2 days in phenol red-free RPMI media with 10% charcoal stripped FCS, 2 mM glutamine, 0.1 mg/mL streptomycin, 100 IU/mL penicillin (Sigma, St Louis, MO) and 2.5 µM β mercaptoethanol (pH 7.4) at 37°C. 0.3 million cells were added to each well which were coated with poly-D-lysine. The Seahorse XF Cell Mito Stress Test kit was used according to the manufacturer's protocol except that FCCP concentrations of 0.5 and 1.0 µM were used. After the analysis, the cell count was estimated using the LSR II cytometer. The OCR and ECAR values were normalized with blank and cell numbers using Wave Desktop software (Agilent Technologies).

4.9 TaqMan qPCR (I)

Total RNA was isolated using RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized with Super Script VILO (Invitrogen) by following the manufacturer's instructions. PROBE FAST ABI Prism 2X qPCR Master Mix (Kapa Biosystems, Woburn, MA) and ABI 7900 HT

thermocycler were used. Primer oligos (**Table 2**) were purchased from Oligomer (Helsinki, Finland). Hprt (Applied Biosystems) was used as endogenous control gene to normalize. Relative gene expression quantification was calculated using $2^{-\Delta\Delta Ct}$ (Pfaffl, 2001).

Table 2: Primers used in the study (publication I)

Gene name	Primer1 Primer2 Probe
Esr1	5'- ccccagggtgatatgaactc -3' 5'- ttgcggaacacattcctgt -3' #93
Hprt	Applied Biosystems

4.10 Immunoblotting (I, II)

Whole cell extracts were isolated using Triton X-100 lysis buffer (50 mM Tris-HCl, pH 7.5, 0.5% Triton X-100, 150 mM NaCl, 5% Glycerol and 1% SDS) supplemented with proteinase and phosphatase inhibitors (both from Roche, Indianapolis, IN). The concentration of protein was estimated with a detergent-compatible protein assay kit (Bio-Rad, Hercules, CA). Samples were boiled with 6X loading dye (330 mM Tris-HCl, pH 6.8, 170 μM bromophenol blue, 30% Glycerol, 330 mM SDS, 6% β-Mercaptoethanol) for 5 min prior loading on to the gel. 30-50 μg of protein was separated on precast 4-15% gels (Biorad). After the gel run, the protein was transferred onto PVDF ((Bio-Rad, Hercules, CA) membranes. The primary antibodies used in the study are listed in **Table 3**. The secondary antibodies used in the study are anti-rabbit (eBioscience, 18-8816-33), anti-mouse (Santacruz, SC-2005), and anti-rat (Santacruz, SC-2956). The protein bands were detected using the Pierce ECL Western blotting substrate (Thermo Scientific, Rockford, IL) enhanced chemiluminescent kit. Gels were imaged with a Chemidoc instrument (Bio-Rad, Hercules, CA). Densitometry or quantification of proteins was performed with Image lab software (Bio-Rad, Hercules, CA).

Table 3: Primary Antibodies used in the study

Name	Company
NFAT1	Cell Signaling (5861S)
STAT5	Cell Signaling (9863S)
FOXP3	Cell Signaling (12653S)
CDK1	Cell Signaling (4688)
ZAP70	BD (Z24820)
Actin-β	Sigma (A5441)
PSMB5	Cell Signaling (12919)
VIM	Cell Signaling (5741)
ENO3	Sigma (AB1)
FOXO1	Immunoway (YM1279)
SMYD3	Abcam (ab127689)

4.11 Transcriptome profiling (I, II)

4.11.1 Sample preparation (I, II)

Naïve ERα$^{fl/fl}$ and CD4-cre ERα$^{fl/fl}$ T ($CD4^+CD44^{lo}CD62L^{hi}CD25^-$) cells were injected separately to Rag$^{-/-}$ mice to elicit colitis. After 10 weeks of colitis induction, $CD4^+$ T cells were FACS sorted from splenocytes of recipient Rag$^{-/-}$ mice. Total RNA was isolated with an RNeasy kit (Qiagen, Hilden, Germany). RNA sequencing (RNA-Seq) libraries were prepared with the Illumina TruSeq RNA sample preparation protocol and sequenced on a HiSeq 2000 machine. Libraries were prepared and sequenced at Finnish Functional Genomics Centre (FFGC), Turku Centre for Biotechnology.

4.11.2 Data analysis (I, II)

RNA-Seq data analysis for the ERα study was performed at BGI Genomics (New Territories, Hong Kong). Briefly, the read quality was assessed with FastQC (0.11.3). Sequencing reads (50 bp single-end) were cleaned to remove adaptors, unknown bases and reads of low quality. After filtering and cleaning, the HISAT/Bowtie2 tool was used to map against a reference genome. Gene expression was determined with the RSEM quantification software. *p-values* for differentially expressed genes were calculated with the Poisson distribution method. Corrected *p*-values were obtained using Bonferroni correction.

RNA sequencing analysis for the proteomic study was performed by bioinformaticians from Aalto University, Helsinki in the following manner. Sequencing reads (100 bp-paired end) were cleaned. Filtered reads were aligned to UCSC mouse transcriptome from Illumina's iGenomes collection and mm10 mouse reference genome using TopHat (version 2.0.14). HTSeq-count (Anders et al, 2014) with options stranded false, feature type exon, sorted by name and mode union were chosen to generate read counts. EdgeR (Robinson et al, 2010) analysis was implemented to identify differential gene expression. Gene-wise statistical tests were performed by fitting the negative binomial generalized log-linear model to the read. Genes with more than 1 cpm (count per million) at least in four samples in the analysis were considered.

4.12 Proteome profiling (II)

4.12.1 Sample preparation (II)

Th17 cells were cultured for 3 days and iTregs were cultured for 10 days along with their respective paired Th0 cells. Cells were re-suspended in lysis buffer (0.5% (v/v) NP-40, 150 mM NaCl and 50mM Tris-HCl) supplemented with proteinase and phosphatase inhibitors (both from Roche, Indianapolis, IN). Sonication was performed for 5 min to completely lyse the cells. Cold acetone (5:1) was added to the lysate to precipitate the proteins (overnight at -20 ˚C). Samples were denatured by addition of 8M urea. Protein concentration was estimated using the BioRad DC kit. An equal amount of protein was taken for further processing of the sample. The reduction reaction was carried out with dithiothreitol at 37°C for 1h and alkylation with iodoacetamide in darkness at room temperature for 30 min. Protein was digested with modified trypsin (Promega sequencing grade) at 37°C at a ratio of 1:30 (trypsin/protein) for overnight (16–18 h). To quench the trypsin activity, digested samples were acidified by adding 10% trifluoroacetic acid (TFA). Desalting of peptides samples was carried out with C18 tips (OMIX, Agilent), which were conditioned with 0.1 % TFA. Equilibration was done twice pipetting with 50:50 (acetonitrile/water). Samples were aspirated 10 times to improve binding of peptides with resin bed. The tip was rinsed twice with 0.1% TFA to get rid of salt. Peptides were eluted in 80% ACN+0.1% Formic acid. HiPPR Detergent Removal Spin Column

Kit (Thermo Scientific) was used according to manufacturer's recommendations. Briefly, 50ul of HiPPR Detergent Removal Resin slurry added to spin column. The column was washed thrice with 25 mM ammonium bicarbonate buffer. 50 ul of sample peptides in ammonium bicarbonate buffer was added to the spin column with detergent removal resin and incubated for 10 mins. Spin column was inserted into fresh microfuge tube to collect detergent-free peptide sample. The peptides were concentrated in speedvac to evaporate buffer. The sample was reconstituted in 2% formic acid and 2% acetonitrile and concentration were determined with NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). The samples were diluted such that 200 ng of peptides were loaded for each LC-MS/MS analysis.

4.12.2 MS run and search (II)

Peptides were initially loaded on a C18 trapping column (100 µm x 20 mm) and subsequently separated on a C18 column (75 µm x 150 mm, 200 Å 5 µm, Dr. Maisch, GmbH, Ammerbuch, Germany). The mobile phase constitutes a binary mixture of water and acetonitrile alone with formic acid. MS analysis were performed using a Q Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to a nano-flow UHP-LC system (Easy-nLC1200, Thermo Fisher Scientific). Peptides were separated from 5% to 35% of solvent B in 85 min at a flow rate of 300 nl/min. A data dependent acquisition method with higher-energy collisional dissociation (HCD) fragmentation of top ten most intense ions in survey scan of mass range 300-1800 m/z were used. The tandem mass spectra were acquired automatically by using Thermo Xcalibur software (Thermo Fisher Scientific). Samples were run in triplicate in a randomized manner. The MS raw files were analyzed by the MaxQuant software (1.5.3.30) and peak lists were searched against the mouse UniProt FASTA database (downloaded on 18.1.2016) using the Andromeda search engine integrated into the MaxQuant software. Carbamidomethylation (C) was set as a fixed modification, methionine oxidation, and N-terminal acetylation as variable modifications. Peptide and protein false discovery rate (FDR) was set to 1%. The match between run options was activated to transfer the identification across the samples.

4.12.3 Downstream bioinformatic analysis (II)

Protein groups from MaxQuant output identified only by site or labeled as potential contaminants were omitted from analysis. Medians of technical replicates were calculated followed by logarithm scale transformation. The values were subjected to quantile normalization. The Limma package in Bioconductor (Ritchie et al, 2015) was used to perform pairwise moderated t-test comparison with Th17 versus Th0 and iTreg versus Th0, and unpaired comparison with Th17 versus naïve $CD4^+$ T cell, iTreg versus naïve $CD4^+$ T cell and Th17 versus iTreg cells to identify differentially expressed proteins. False discovery rates (FDR) were obtained using Benjamini-Hochberg multiple correction. Proteins with FDR <0.05 were considered as differentially expressed proteins. Proteins with intensity values detected in only one of the conditions (for example IL-17F, FOXP3 etc.) were considered as selectively expressed without applying any statistics. A protein was considered to be expressed only if it was detected at least in two biological replicates.

4.13 Cytoscape network (II)

Differentially expressed proteins from Th17 vs. iTreg were used in network analysis. Protein-protein interactions were obtained by querying the proteins in stringAPP (1.3) in Cytoscape (3.6) software. To obtain edges a string confidence score of 0.7 was used. Network analyzer was used to obtain degrees of the node. Networks were displayed in hierarchical and organic layouts from the yFiles layout algorithm.

4.14 Data availability (I, II)

The RNA-Seq data from the ERα study was deposited at the NCBI Gene Expression Omnibus (GEO) with the accession number GSE107284. The mass spectrometry proteomics data was deposited in the ProteomeXchange Consortium via the PRIDE (Vizcaíno et al., 2016) and can be accessed via dataset identifier PXD007826. The RNA-Seq data for Th0, Th17, and iTreg can be found at GEO with accession number GSE 104152.

4.15 Statistical analysis (I, II)

Statistical analyses were made using a Student's *t* test or two-tailed Mann-Whitney test, where appropriate. Error bars correspond to mean ± SEM, unless otherwise indicated.

5 RESULTS AND DISCUSSION

5.1 Role of Estrogen receptor alpha in autoimmune colitis (I)

5.1.1 Estrogen receptor alpha contributes to autoimmune colitis

To investigate the role of estrogen receptor alpha in autoimmune settings, an adoptive T cell transfer colitis model was set up by injecting naïve T cells from littermate control and CD4-creER$\alpha^{fl/fl}$ mice to $Rag^{-/-}$ mice, which were monitored for 9 weeks. After the 4th week, $Rag^{-/-}$ mice injected with ERα deficient T cells kept gaining weight, whereas the mice that received control T cells developed a wasting disease. Consistently, ELISA quantification of serum pro-inflammatory cytokines (such as TNF-α, IL-6, IFN-γ, IL-12p70 and MCP-1) showed increased levels in the mice that received control T cells, compared to mice received CD4-creER$\alpha^{fl/fl}$ T cells. Reduced infiltrations of T cells in the colon were observed in mice that received CD4-creER$\alpha^{fl/fl}$ T cells. Additionally, histological assessment of colonic inflammation confirmed the lower severity of inflammation. Taken together, these results indicate that T cell expressed estrogen receptor is necessary for induction of experimental autoimmune colitis in mice.

5.1.2 Estrogen receptor alpha in T cell survival and proliferation

To identify the molecular mechanism of estrogen receptor alpha in T cells, we performed RNA-Seq of littermate control and ERα deficient colitogenic T cells isolated from $Rag^{-/-}$ spleens after induction of colitis. Transcriptome profiling revealed the enrichment for genes responsible for T cell receptor signaling suggesting that ERα is involved in T cell activation. The expressions of a number of genes responsible for T cell activation, such as Zap70, Lat, Itk, Plcg1, Cd69, Prkcq, Lck, Fyn, Nfatc2, Rasgrp1 and Grap, were reduced and the expression of several genes responsible for cancer (Ptpn6, Pak1) and cell adhesion (Icosl, Itgam) were increased in ERα deficient cells compared to littermate control T cells. To verify the contribution of ERα to *in vitro* T cell activation, naïve T cells isolated from ER$\alpha^{fl/fl}$ and CD4-creER$\alpha^{fl/fl}$ were activated

with varying concentrations of anti-CD3 and anti-CD28. Early and late activation markers (CD69 and CD25, respectively) were quantified with flow cytometry. Clearly, CD69 levels were reduced in CD4-creER$\alpha^{fl/fl}$ cells in comparison with control. Immunoblot analysis of proteins such as NFAT1, STAT5, CDK1 and ZAP70 showed that their levels are reduced in ERα deficient T cells. Cell survival was estimated with annexin-PI staining, and a reduced percentage of apoptotic cells was observed in CD4cre-ER$\alpha^{fl/fl}$ T cells. Apoptotic regulator protein Bcl2 was detected with flow cytometry. Higher levels of Bcl2 were observed in CD4-creER$\alpha^{fl/fl}$ T cells. Bcl2 regulates cell death by preventing the loss of outer mitochondrial membrane integrity. Hence, we measured the mitochondrial membrane potential with Tetramethylrhodamine, ethyl ester (TMRE) dye by *in vitro* culturing ER$\alpha^{fl/fl}$ and CD4-creER$\alpha^{fl/fl}$ $CD4^+$ T cells. TMRE is a cationic, lipophilic dye that accumulates in active mitochondria, we observed upregulation of TMRE in CD4-creER$\alpha^{fl/fl}$ T cells indicating the presence of more active mitochondria in CD4-creER$\alpha^{fl/fl}$ T cells.

Cell trace violet staining was used to assay proliferation of T cells, ER$\alpha^{fl/fl}$ and CD4-creER$\alpha^{fl/fl}$ cells that were cultured *in vitro* for 4 and 5 days. Dilution of dye indicates each generation of dividing T cells. A defect in proliferation was observed clearly in ERα deficient T cells. An *in vivo* T cell proliferation assay was performed by injecting CFSE labeled WT ($CD45.1^+$ C57BL6/J) and $CD45.2^+$CD4-creER$\alpha^{fl/fl}$ naïve T cells into $Rag1^{-/-}$ recipient mice. 10 days after injection, cells were isolated from spleen/blood to determine the dilution of dye with flow cytometry. WT T cells proliferated more compared to $CD45.2^+$CD4-creER$\alpha^{fl/fl}$ cells. These data indicate that ERα is required for T cell proliferation and expansion.

5.1.3 Estrogen receptor deficient T cells are more sensitive to iTreg phenotype

Naïve T cells isolated from ER$\alpha^{fl/fl}$ and CD4-creER$\alpha^{fl/fl}$ mice were polarized to iTregs in the presence of anti-CD3, anti-CD28, IL-2 and TGF-β1 for three days. In CD4-creER$\alpha^{fl/fl}$ T cell culture, an increased percentage of Foxp3 cells was observed compared to ER$\alpha^{fl/fl}$. On the other hand, retroviral over expression of ERα in naïve cells under the iTreg polarizing conditions led to a decrease in levels of Foxp3. Addition of estrogen ligand (17β-Estradiol, E2) did not increase the percentage of $Foxp3^+$ cells in CD4-creER$\alpha^{fl/fl}$ T cell culture. Additionally, RNA-Seq analysis of

colitogenic T cells isolated from $Rag^{-/-}$ mice injected with CD4-creER$\alpha^{fl/fl}$ cells showed increased levels of iTreg signature genes such as Foxp3, TGF-β1, Dnmt1, Satb1 and Tcf1 expression. Also, elevated levels of Foxp3 were observed in T cells isolated from spleen and lamina propria of $Rag^{-/-}$ mice injected with CD4-creER$\alpha^{fl/fl}$ cells. Collectively, these results indicate that ERα deficiency in T cells favors an iTreg phenotype.

5.1.4 Discussion

Autoimmune diseases show higher predominance in females. The contributing factors are environmental, X-chromosome inactivation, fetal microchimerism and sex hormones (Selmi et al., 2012). Estrogen hormones exert a biphasic effect in female autoimmunity; it shows protective action in multiple sclerosis and deleterious role in system lupus erythematosus. Reports have shown that estrogen regulates disease severity in IBD patients (Ferguson et al., 2008; Kane and Reddy, 2008; Riis et al., 2006). In an IBD disease model carried out with HLA-B27 transgenic rat, estrogen therapy reduced diarrhea and intestinal inflammation (Harnish et al., 2004).

In the first part of this thesis, T cell specific deletion of ERα was found to result in the attenuated T cell mediated inflammation and impaired proliferation whilst enhancing T cell survival and differentiation to iTreg cells. In our study, we focused on the adoptive transfer colitis model that is caused predominantly by Th1 and Th17 cells. The protective phenotype of estrogen receptor deficiency observed in our study can be explained by a defect in proliferation of CD4-creER$\alpha^{fl/fl}$ T cells and also by their propensity to skew the differentiation towards iTreg cells. Gene expression analysis of CD4-creER$\alpha^{fl/fl}$ T cells isolated from the colitogenic environment showed altered levels of specific candidate genes known to be important for T cell activation, i.e. Zap70, Lat, Itk, Plcg1, Cd69, Prkcq, Lck, Fyn, Nfatc2, Rasgrp1 and Grap. Consistently, we observed a defect in activation when cells were cultured with varying concentrations of anti-CD3 and anti-CD28 *in vitro.* In proliferation assays, CD4-creER$\alpha^{fl/fl}$ cells stained with cell trace violet showed impaired proliferation. These observations strongly suggest the role of ERα in cell activation and proliferation.

Bcl-2 family members show both prosurvival (Bcl-w, Bcl-XL. and Mcl-1) and proapoptotic (Bak, Bax, and Bok) responses (Popgeorgiev et al., 2018). Bcl2 acts as pro-survival protein and also has cell cycle inhibitory function (Quinn and Richardson, 2004), which is consistent with our observation *in vitro* cultured CD4-creER$\alpha^{fl/fl}$ cells exhibited upregulated Bcl2, enhanced survival ability and also reduced levels of Cdk1 (a cell cycle related protein). The reduced levels of Cdk1 in CD4-creER$\alpha^{fl/fl}$ T cells might be because of increased levels of Bcl2, as nuclear Bcl2 blocks cell cycle entry (G1-to-S transition) by inhibiting Cdk1 and E2F activity (Vairo et al., 2000). Interestingly, ERα is also present in mitochondria and exerts the actions of E2 (Chen et al., 2004). Like ERα, Bcl2 proteins can also be found in the mitochondria. Bcl2 protein localized in outer mitochondrial membrane prevents apoptosis by inhibiting proapoptotic proteins (Bak, Bax) and controls inner mitochondrial membrane permeability. TMRE dye can be used to determine membrane potential and as an active mitochondria indicator. We observed the higher proportion of $TMRE^{hi}$ cells in CD4-creER$^{fl/fl}$ cell cultures. Together, these data show that ERα depletion in T cells upregulated Bcl2, reduced apoptosis and increased cell survival.

Suboptimal T cell activation is needed for induction of iTreg cells (Haxhinasto et al., 2008). TCR signaling regulates the Foxp3 expression via PI3K/Akt/mTOR signaling (Sauer et al., 2008). The Akt-mTOR pathway negatively regulates Foxp3 expression during iTreg differentiation (Delgoffe et al., 2011). Additionally DNMT1 also negatively regulates Foxp3 expression (Josefowicz et al., 2009). In our study, the decreased levels observed for proteins such as Akt1, mTOR and DNMT1 might be the reason for increased levels of Foxp3 in ERα deficient iTreg cells. ERα regulates the transcription of its target genes in a ligand dependent and ligand independent manner. Addition of E2 to ER$\alpha^{fl/fl}$ cells increased Foxp3 expression and deletion of ERα abrogated the E2-dependent increase of Foxp3 in CD4-creER$^{fl/fl}$ cells. Consistent with previous reports, estrogens via ERα signaling promote iTreg molecules such as Foxp3, PD-1 and CTLA4, and downregulate the immune responses (Polanczyk et al., 2004, 2007).

ERα regulates the transcription of its target genes by interacting with transcription factors and cofactors (Shang et al., 2000). Estrogens have been observed to upregulate expression of Bcl2 in breast cancer cells via genetic and non-genetic ERα pathways

(Svotelis et al., 2011). In human breast cancer MCF-7 cells, ERα positively regulates DNMT1 expression by binding to its gene promoter (Shi et al., 2012).We observed depletion of ERα in CD4-creER$\alpha^{fl/fl}$ T cells led to reduced expression of Bcl2 and DNMT1 proteins, indicating that ERα might directly or indirectly regulate Bcl2 and DNMT1 levels.

In conclusion, we have identified the important of role of ERα in T cell activation, survival and proliferation. ERα promotes autoimmune inflammation in mouse model of colitis. ERα depleted T cells showed the elevated levels of Foxp3 expression. The observations from our study may have implications for estrogen therapies in autoimmune diseases and also in understanding the pathobiology of autoimmune diseases in females.

5.2 Proteomic profiling of murine Th17 and iTreg cells (II)

5.2.1 Overview of detected proteins in Th17 and iTreg cells

We profiled global proteome changes in Th17 and iTreg cells by applying a shotgun proteomics approach. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was employed to study naive, Th17, iTreg, Th0 paired with Th17 and Th0 paired with iTreg cells that were *in vitro* cultured for 3 days and 10 days. In total, 4287 protein groups were identified in all the subsets using label free quantitative (LFQ) strategy. The detected proteins span six orders of magnitude in expression with coverage from all the cellular compartments. Proteins such as IL2RA, IL2RG, TNFRSF18, ICAM1, ICAM2, ITGAL, ITGB1, ITGB2, ITGB7, CD3E, CD3G, CD2, CD4, CD5, CD6, CD28, CD44, and CD69 that are crucial for T cell activity were identified in all Th cell subsets. Functional group analysis of proteins was carried out with ingenuity pathway analysis (IPA). Enzymes top the list of identified proteins, followed by transporters, transcription factors, peptidases, kinases, transmembrane receptors, phosphatase, ion channel, translation regulator and cytokines.

5.2.2 Protein expression changes in Th17 and iTreg cells

In depth analysis of abundance and differential expression patterns during the differentiation of T cells allows better understanding of the processes involved and also in the development of strategies to perturb the proteins for autoimmune disease treatment and cancer immunotherapy.

Th17 cells: Th17 cells were polarized from naïve $CD4^+$ T cells by culturing in the presence of IL-6, TGF-β1, anti-CD3, anti-CD28, anti-IL-4 and anti-IFN-γ for three days. Proteomic samples were prepared by lysing the cells in NP-40 buffer followed with trypsin digestion. Three biological replicates were analyzed by LC-MS/MS. We identified 1005 statistically significant (false discovery rate, [FDR] < 0.05) differentially expressed (DE) proteins in Th17 vs Th0 comparison. These included 414 upregulated proteins, such as IL17F, RORC, AHR and IKZF3, and 591 downregulated proteins, such as TNFRSF18 (GITR) and TNFRSF4 (OX40). We confirmed the expression changes of selected proteins identified in the LC-MS/MS analyses by flow cytometry and immunoblotting. A consistent expression change of CD101 was detected in Th17 cells with flow cytometry and PSMB5 and NFATC2 were detected with immunoblotting. In the Th17 vs. iTreg comparison, 2040 DE proteins were identified, of which 1067 proteins were more abundant in Th17 cells. DNMT3A, BACH2, JAK3, TCF7, JUNB, LAG3, IL2rg are upregulated in Th17 in comparison with iTreg cells. Interestingly, when compared to Th0 cells the expression of another 19 proteins, such as ETV6, TGM2, and SATB1 were highly expressed in Th17 cells and lowly expressed in iTreg cells. Functional annotation of proteins was performed to categorize DE proteins from the Th17 vs. Th0 comparison showing transmembrane receptor proteins, transcription factors, and cytokines (based on IPA annotation) and metabolic regulators (obtained from KEGG analysis) (**Figure 6**).

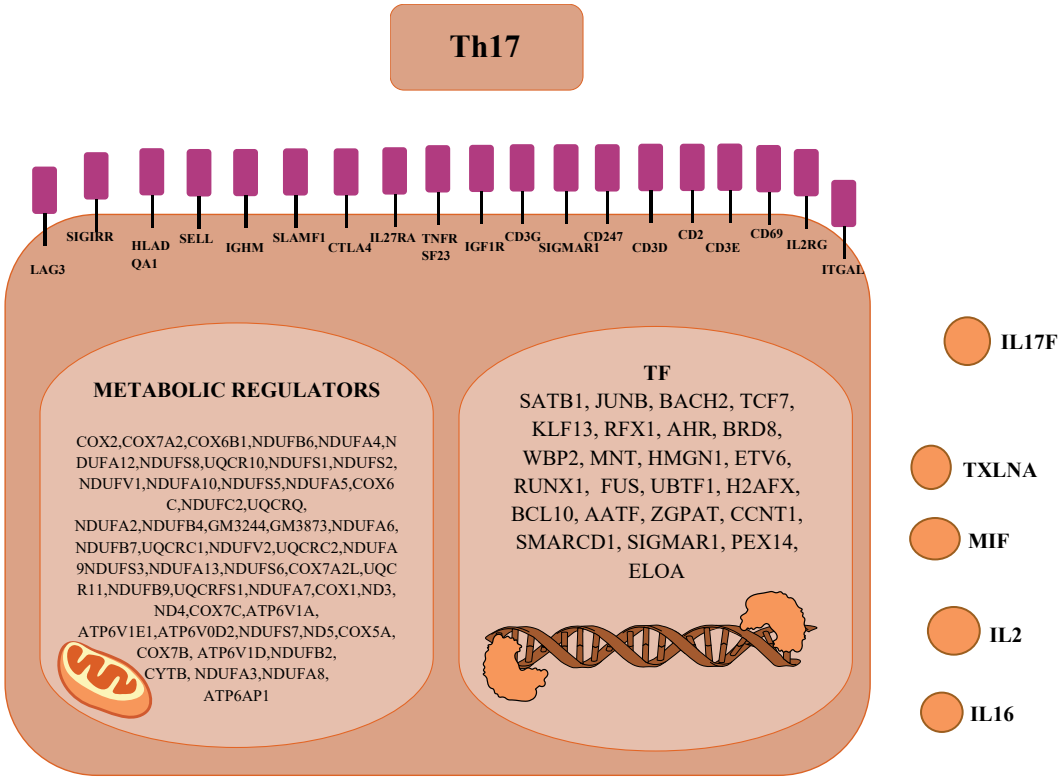


Figure 6: Proteins identified in Th17 cells. Transmembrane receptor proteins, transcription factors (TF) and cytokines are shown (based on IPA annotation). The annotation for metabolic regulators was obtained from KEGG analysis. The representation of DNA is modified from https://commons.wikimedia.org/wiki/File:Difference_DNA_RNA-EN.svg. The mitochondrion is modified from https://commons.wikimedia.org/wiki/File:Animal_cell_structure_en.svg

iTreg cells: iTreg cells were polarized from naïve $CD4^+$ T cells by culturing in the presence of IL-2, TGF-β1 for 10 days. Proteomic samples were prepared by lysing the cells in NP-40 buffer followed with trypsin digestion. Three biological replicates were analyzed by LC-MS/MS. Comparison of iTreg with Th0 cells accounted for 675 statistically significant (FDR < 0.05) differentially expressed proteins that include 308 upregulated and 367 downregulated proteins. IKZF4, RUNX3, ITGAE, GNG2 and VIM were observed to be up-regulated and SATB1 (previously reported that SATB1 is abundant in Treg cells (Beyer et al., 2011)) and CD69 were down-regulated in expression. In the comparison of Th17 vs. iTreg cells, out of 2040 DE proteins, 973

proteins (including TRAF6, TGFβ1, CD44, IL2RA, ITGAE and VIM) were highly expressed in iTreg cells. We further validated the protein expression changes of SMYD3, VIM, FOXO1, and ENO3 in iTreg cells with immunoblotting. Functional annotation of proteins was performed to categorize the DE proteins from the Treg vs. Th0 comparison, highlighting transmembrane receptor proteins, transcription factors, and cytokines (based on IPA annotation) and metabolic regulators (obtained from KEGG analysis) **(Figure 7)**.

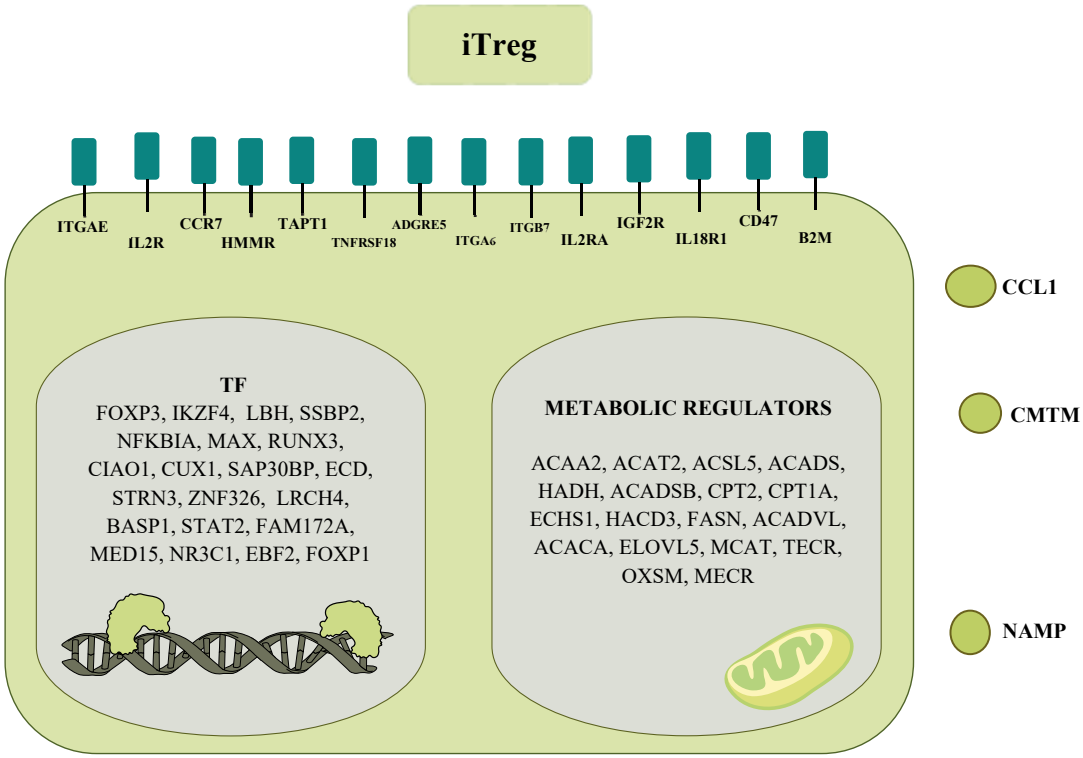


Figure 7: Proteins identified in iTreg cells. Transmembrane receptor proteins, transcription factors (TF) and cytokines are shown (based on IPA annotation). Metabolic regulators are obtained from KEGG analysis. The representation of DNA was modified from https://commons.wikimedia.org/wiki/File:Difference_DNA_RNA-EN.svg. Mitochondrion is modified from https://commons.wikimedia.org/wiki/File:Animal_cell_structure_en.svg

5.2.3 Pathways and networks of Th17 and iTreg cells

To assess the biological functions of DE proteins, we performed KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis. Th17 cells showed statistically significant (adjusted p value < 0.05) enrichment for spliceosome, ribosome and oxidative phosphorylation, and iTreg cells demonstrated significant (adjusted p value < 0.05) enrichment for fatty acid metabolism, aminoacyl-tRNA biosynthesis, alanine, aspartate and glutamate metabolism pathways. Furthermore, GSEA (Gene set enrichment analysis) was carried out on the proteins identified with KEGG analysis to obtain the meaningful networks. The STRING database was used to obtain protein-protein association networks. Application of network analyses helps in better understanding the biological pathways and signaling mechanism involved in immune processes (Amit et al., 2011).

Transcriptome profiling was also performed for Th0 and Th17 cells prepared in similar way as the proteomics cultures. The results of the proteomic with transcriptomic analysis were overlaid (compared) to obtain novel insights regarding the molecules involved in differentiation. 963 proteins with corresponding transcripts in Th17 cells were overlaid with 1004 detected transcripts, 399 were observed commonly in both technologies. Out of 399 proteins, 139 are upregulated in both proteome and transcriptome detections (hits such as Il17f, Rorc and Ahr) and 145 are downregulated in both proteome and transcriptome detections (Tnfrsf18, Cd44, Il2rg and Iigp1).The remaining 115 hits showed inverse correlation with 45 proteins (upregulated in protein, downregulated in mRNA) and 70 proteins (down regulated in protein, upregulated in mRNA).

5.2.4 The role of vimentin in iTreg cells

In our proteomic screen, we observed that vimentin protein is upregulated in iTreg cells compared to Th0 cells. Vimentin belongs to the group of cytoskeletal intermediate filament proteins. It provides mechanical support to the cell membranes and helps in maintaining the organelles and nucleus in a defined place within the cell (Eriksson et al., 2009). In order to study the effect of vimentin on Treg cell differentiation, we polarized the naïve wildtype $CD4^+$ T and vimentin deficient ($Vim^{-/-}$)

$CD4^+$ T cells to iTreg condition for 3 days. We did not observe any change in expression levels of Foxp3 from the polarized wildtype and $Vim^{-/-}$ iTreg cells. To further study the effect of VIM on the cytokine TGF-β and IL-2 induced Foxp3 expression, the naïve $CD4^+$ T cells isolated from $Vim^{-/-}$ and wild type mice were cultured with TGF-β alone, IL-2 alone, and combination of TGF-β and IL-2. We observed reduced levels of Foxp3 in $Vim^{-/-}$ T cells cultured alone with TGF-β. Interestingly, addition of IL-2 to TGF-β culture in $Vim^{-/-}$ T cells restored the Foxp3 levels similar to wild type controls. Furthermore, to assess the TGF-β response on vimentin, wild type naïve T cells were polarized for three days with and without TGF-β inhibitor (LY2109761). Addition of TGF-β inhibitor during polarization of naïve cells to iTreg cells restricted both vimentin and Foxp3 expression, indicating that TGF-β mediates the induction of both vimentin and Foxp3.

5.2.5 Discussion

The advent of such technologies as microarrays and next generation sequencing, along with the human genome project, has accelerated the application of holistic and systematic measurement of cellular molecules such as RNA (transcriptomics), and DNA (genomics). However, transcript level do not always correspond reliably with the abundance of protein (Maier et al., 2009). Proteins that respond to environmental stimuli often do not show changes at mRNA transcript level (Hillenmeyer et al., 2008). In our study, we made advantage of the "shotgun" proteomics approach that enabled high throughput identification of proteins from complex mixtures with tandem MS (LC-MS/MS). This also provided reproducible and detailed proteomes of the cells studied. Unlike chemical labeling procedures, label free quantification facilitates better accuracy and a wider dynamic range of protein quantification. Sample preparation is a critical step in MS based proteomic study; the application of NP-40 detergent lysis buffer has improved the ability in solubilizing large fractions of membrane proteins. We identified 317 and 312 plasma membrane proteins in Th17 or iTreg cells which is comparable with study that identified 229 surface proteins with PAL (periodate oxidation and aniline-catalyzed oxime ligation) combined LC-MS/MS approach from naive unstimulated and activated human $CD4^+$ T cells (Graessel et al., 2015). The

study of membrane proteins or receptors on T cells has gained significant importance as they can be targeted in tumor immunotherapy (Pardoll, 2012).

Genetic data alone does not provide insight into mechanism of diseases; therefore we determined the protein abundance levels during the course of differentiation of T cells. Proteins were detected with a variety of different functions, including transcription factor, enzymes, kinases and phosphatases. The functional annotation of the DE proteins showed enrichment for metabolism. Recent studies have indicated the importance of metabolic reprograming in determining the T cell fate (Buck et al., 2015; MacIver et al., 2013). T cell activation and differentiation causes metabolic rewiring of the cell. Naïve T cells observe oxidative phosphorylation (OXPHOS) mode of energy source and then shift to glycolysis in Th17 cells and fatty acid oxidation in Treg cells during differentiation (Almeida et al., 2016; MacIver et al., 2013; McKinney and Smith, 2018). In our comparison of Th17 with Th0, we observed Th17 switch towards oxidative phosphorylation. However, we also observed upregulation in proteins related to glycolysis such as HK2, DLD, PGK1, PFKL, LDHB, PGAM2, LDHA, PKM, ALDOA, PGM1, ALDOC, TPI1 and MINPP1. Hexokinase 2 (HK2) is a key rate-limiting enzyme in glycolysis. In iTreg cells, high levels of ENO3 - a glycolytic enzyme, and CPT1A- an important rate-limiting enzyme of fatty acid oxidation were observed. Overall the role of proteins involved with metabolism is not completely understood in respect to Th17 and iTreg cell differentiation.

As a target of further studies, it would also be interesting to profile the proteome of Th17 and iTreg cells at early (12 hours, 24 hours) and late (day 5, day 7) time points to study the dynamic behavior of their proteomes. This will distinguish the co-expressed proteins that are involved in the determination of cell fate, expressed in the same sub-cellular compartments, transitioning of energy sources during the differentiation to effector cells, and delay between transcription and translation.

Comparisons of proteomic and transcriptomics data also provide non-overlapping information that can be further explored to obtain insights in to the regulation in T cells. We observed 29.5 percent RNA and protein concordance that is consistent with previously reported studies. Pearson correlation coefficient observed in bacteria 0.47, yeast 0.34, and in multi-cellular organisms 0.46 (Abreu et al., 2009; Foss et al., 2011;

Ghazalpour et al., 2011). In our study, 29.5% of concordance between proteomic and transcriptomic profiling indicates that variance in protein expression changes can be accounted by changes in mRNA expression levels and remaining 70.5 percent can be explained because of technical and biological factors. The technical factor, such as choice of proteomic strategy to measure protein levels is limited to only high abundant proteins. The biological factors that also influence the concordance are translational efficiency, folding of protein, alternative splicing, transport, localization, translation regulation and protein degradation.

The proteomic data obtained from this study can be compared with human data to obtain novel insights in terms of T helper cell differentiation and developing strategies for treatment of human autoimmune diseases. However, the immunological differences between human and mice have been previously reported (Mestas and Hughes, 2004; Seok et al., 2013). Comparative transcriptomic analysis performed in murine and human Th17 cells revealed that 307 orthologous genes pairs are similarly regulated and a positive correlation of 0.68 was reported in between both species (Tuomela et al., 2016). Unlike murine Th17 differentiation, human Th17 cells require IL-1β for Th17 polarization indicating the difference in differentiation mechanism between two species (Acosta-Rodriguez et al., 2007). Additionally, segmented filamentous bacteria (SFB) induces Th17 cell in the intestines of mice (Ivanov et al., 2009), the differences in distribution and colonization of SFB in gut of both species indicate the requirement of different cytokines for Th17 induction. $CD25^{+}$ regulatory T cells exhibit suppressor function, Foxp3 is a master transcription regulator in Treg cells. Ectopic expression of Foxp3 led to suppressor activity in effector cells (Fontenot et al., 2003). Additionally, Foxp3 is highly conserved between human and mice (Lal et al., 2009) and ectopic expression of human Foxp3 in murine cells also led to suppressor activity. *In vitro* cultured human iTreg cells are suppressive in function, but the *in vivo* suppressive ability of human iTreg cells has been a controversial issue (Schmidt et al., 2016). This aspect of iTreg cells should be considered before developing any *in vivo* strategies related to suppressive function in humans.

To demonstrate the strength of our data as a resource, we functionally characterized the protein vimentin, as a regulator of TGF-β induced iTreg differentiation. Vimentin is a cytoskeleton protein and functions as scaffold for signal transduction events (Eriksson

et al., 2009). Firstly, we observed that expression of vimentin protein is induced in iTreg cells in TGF-β dependent manner. The downstream signaling of TGF-β such as in the Smad pathway might be involved in upregulation of vimentin expression. It is known that TGF-β induces Foxp3 expression (Zheng et al., 2002). Consistently, deletion of vimentin and polarization of cells in the presence of TGF-β showed the reduced Foxp3 expression. IL-2 and TGF-β are critically required for $Foxp3^+$ Treg cells (Horwitz et al., 2008). Moreover, Treg dysfunction is restored with the addition of IL-2 cytokine. It might be because IL-2 controls the stability of Foxp3 expression in TGF-β induced $Foxp3^+$ T cells (Chen et al., 2011).

In summary, our results provide a comprehensive overview of protein abundance during the differentiation of Th17 and iTreg cells. Our study leads to better understanding of protein dynamics and molecular mechanism involved in effector cells phenotype. The dataset is valuable for developing novel therapeutic approaches in Th17/iTreg mediated diseases.

6 CONCLUSION AND FUTURE DIRECTIONS

The work presented in this thesis was focused on identifying the novel modulators of T helper cell differentiation and characterizing the role of estrogen receptor alpha protein as a contributor of autoimmunity.

The study on ERα addresses the role of ERα protein in colitis autoimmune disease model. Due to the limitations – on performing genetic perturbations in humans, the experiments were performed on genetically modified mice. Nevertheless, the mechanism and regulators identified in the study can be extrapolated to understand the mechanism or target the T helper cells in human autoimmune diseases. Moreover, to further characterize the emerging model humanized mice can be used to obtain knowledge that can be translated to human (Rongvaux et al., 2014).

ERα is a transcription factor that exerts its action by binding to DNA elements in target genes. To identify the binding sites (promoters or enhancers) of ERα in target genes, ChIP-Seq analysis should be performed in T cells. However, the limiting factor has been the lack of a suitable antibody to establish the binding sites of ERα with high confidence. ERα affects gene transcription through interacting with other transcription factors. Pulldown assays of ERα to identify interacting partners of ERα will also help in understanding the molecular role of ERα in influencing the expression of Foxp3 and Bcl2 in T cells. It is already known that each isoform of ERα has an individual role in cancer cells (Al-Bader et al., 2011). In human T cells, we observed the expression of three isoforms of ERα and it would be interesting to dissect the function of different ERα isoforms in mouse T cells. Estrogens have been implicated in SLE disease and also to influence cytokine production (Kassi and Moutsatsou, 2010). Alterations in the phenotype of Tfh cells has also been observed in SLE patients (Blanco et al., 2016). Hence, it would be interesting to explore the effect of estrogen hormone and ERα deficiency in Tfh cells in SLE animal models. Additionally, it would be interesting to identify the T helper differentiation signaling pathways affected in the presence of E2.

This proteomic analysis of Th17 and iTreg cells has provided a catalog of proteins that may participate in autoimmune disease. The identified molecules could lead to the

development of therapeutic targets for autoimmunity and tumor immunity. The selected candidates in human Th17/iTreg cell differentiation can be perturbed with siRNAs or CRISPR-cas9 methodology to identify the potential biomarkers/therapeutic targets. The integration of proteome information with multi-dimensional biological information, such as phospho-proteomics and bisulfite sequencing, can be performed in order to identify crucial regulators involved in cell signaling during T cell differentiation. Application of novel computational pipelines or algorithms may help in finding the novel regulators of Th17 and iTreg differentiation. The lower abundant proteins can be identified by improving the depth and resolution of protein identification by fractionating the samples before LC-MS/MS for analysis. This would help in determining the low abundant proteins that might have a crucial role in lineage commitment. Such a proteomic study could be performed by culturing the naïve cells with the IL-2, IL-6 and TGF-β cytokines individually and in combination. TGF-β is essential for both Th17 and iTreg cells and an in depth study might help in understanding how the balance can be achieved by TGF-β and IL-6 in favor of Th17 cells.

In summary, the data from these studies could be used to improve the understanding of autoimmunity in females, especially in the context of the estrogen hormone. The DE proteins highlighted from the comparison of Th17 and iTreg cells might prove to be potential targets for the treatment of autoimmune diseases.

ACKNOWLEDGEMENTS

I sincerely thank Dr. Zhi Jane Chen for guiding and supporting my PhD study. I am also grateful to her for providing me with opportunity to work on challenging projects and amazing field of T lymphocytes biology and autoimmune diseases. Her scientific competence and never ending ideas have been always admirable. I acknowledge Dr. Riitta Lahesmaa for providing an excellent working environment for immunology research at Turku Center for Biotechnology.

Turku doctoral program for molecular medicine (TUDMM) is duly noted for providing financial support and pertinent course work, aiding in the successful completion of my Ph.D. I am grateful to the supervisory board members Dr. David Hawkins for lending positive feedback and support and Professor Bing Li for his constructive criticism and comments. I would also like to acknowledge Dr. Harri Lähdesmäki for excellent discussions and valuable comments on the proteomics study.

I would like to thank co-authors Wendy Watford, Fang Cheng, Tamas Nagy, Emrah Yatkin, Kalervo Väänänen, Anne Rokka, David R. Goodlett and John E. Eriksson. The reviewers of my thesis Dr. Marko Pesu and Dr. John Andersson for their indispensable comments and constructive criticism regarding the thesis.

The PhD journey would not have been easy without these people. Especially Inna Starskaia for all the support and care during the tough times in the lab, help with the experiments, and fun outside and inside the lab. Carlton Xavier for being an amazing friend and room partner who is always hungry (He took Steve Jobs mantra “Stay foolish and stay hungry” seriously). Dhana Prakash Jumbolingam for being an awesome periya Tevidiya, Venkat Subramaniam for the interesting road trips and motivational talks. Ankitha Shetty for all the support, chats, fun trips and outstanding poor jokes (PJs). Obaiah Dirasantha for the support, Upma, Indian hang-outs and especially for creating a Telugu atmosphere and jokes. Subhash Tripathi and Santosh Bhosale for being a family to me away from home. Moin Khan’s invaluable help in getting the initial experiments started and on discussions regarding GLP (Good lab practices). Naresh Jayavelu Doni for providing support and help alongside. Karteik Kanduri for sharing cues on how to successfully complete a PhD and his extensive

knowledge on Data visualization and R programming. Kalyan Pasumarthy anna for coming up with interesting topics to discuss. Mahesh reddy Emani anna for his pep talks and help with microscopy sample preparation. Brigitta Csendes for being an excellent lab colleague during the start of my PhD and introducing me to Hungarian culture, Johanna Tuomisto for hangouts (tips on breathe-in/breathe out☺) that provided much required relief, Tanja Buchacher for sharing tips from her PhD and pushing me to finish my PhD, Ji Tao for discussion on iTreg experiments and his tricks in Western blots and gel preparations. Ubaid Ullah with his humor that takes a while to realize, Karolina Hirvonen with her infectious laugh on the 7th floor, Robert Moulder for his encouraging words and support in proteomic study. Kari Nousiainen for exceptional collaboration in proteomic study. Henna Kallionpaa for her kind words. Omid Rasool for his help. Mikko Konki for sharing office space. Elina Peitilla for her help with Luminex run. Marjo Hakkarainen who's always in high spirits and for sharing interesting stories in cell culture lab. Ponnuswamy Mohanasundaram for sharing his knowledge on vimentin and smile. Other members in ATLAS lab- Bilal Andrabi, Sarita Heinonen, Niina Lietzen, Verna Salo, Essi Laajala, Soile Tuomela and Senthilkumar Palanisamy.

Andhra batch-Sai kumar, Balaji (Kota srinivasa rao) and Obaiah ((Babu Mohan) with their humorous stories (Viva Harsha, Amrutham, Finnish ghosts in their room, Chicken curry) and help. Meraj Khan, Umar Butt, Pranshu Sahgal, Neeraj Prabhakar, Alexey Sarapulov, Arun Venu, Fakhrul Faruque, Srikar Nagelli, Nitin, Vipin Ranga, Farid Ahmad and colleagues from 5th floor for sharing reagents and enthusiasm. Afshan Syeda, Samir Kumar Jagirdar and BIMA batch for fun trips, birthday parties and Indian food. Ramzan ali and John Chen for sharing the TYS apartment and introducing me to various foods and sports. Trang for an amazing and cherishing Paris trip. Sonia Panini for idi rabotaj.

I am thankful for excellent service from CIC especially Adel Ketlin and Jouko Sandholm for sorting T cells. The staff at the Central Animal Laboratory of the University of Turku especially Suvi and Baeta for taking good care of mice. The staff of mass spectrometric unit at the Turku Centre of Biotechnology especially Arttu Heinonen for his exceptional skills in trouble shooting the problems with MS run during protocol and method optimizations. I would like to thank people such as Rafael

Casellas, Franke Lude and Zoltan Fehervari with their fantastic talks for inspiration. Last, but not least the Academy of Finland for funding the study.

My mom, dad and brother for their unconditional support and love and all my relatives for their encouragement. Planet Earth for accommodating me and science for get me going.

Turku, August, 2018

Imran Mohammad

REFERENCES

Abreu, R. de S., Penalva, L.O., Marcotte, E.M., and Vogel, C. (2009). Global signatures of protein and mRNA expression levels. Mol. Biosyst. 5, 1512–1526.

Acosta-Rodriguez, E.V., Napolitani, G., Lanzavecchia, A., and Sallusto, F. (2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat. Immunol. *8*, 942–949.

Aebersold, R., and Mann, M. (2016). Mass-spectrometric exploration of proteome structure and function. Nature *537*, 347–355.

Afkarian, M., Sedy, J.R., Yang, J., Jacobson, N.G., Cereb, N., Yang, S.Y., Murphy, T.L., and Murphy, K.M. (2002). T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. Nat. Immunol. *3*, 549–557.

Agarwal, S., Avni, O., and Rao, A. (2000). Cell-type-restricted binding of the transcription factor NFAT to a distal IL-4 enhancer in vivo. Immunity *12*, 643–652.

Ahmad, Y., and Lamond, A.I. (2014). A perspective on proteomics in cell biology. Trends Cell Biol. *24*, 257–264.

Akimzhanov, A.M., Yang, X.O., and Dong, C. (2007). Chromatin Remodeling of Interleukin-17 (IL-17)-IL-17F Cytokine Gene Locus during Inflammatory Helper T Cell Differentiation. J. Biol. Chem. *282*, 5969–5972.

Al-Bader, M., Ford, C., Al-Ayadhy, B., and Francis, I. (2011). Analysis of estrogen receptor isoforms and variants in breast cancer cell lines. Exp. Ther. Med. *2*, 537–544.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). Molecular Biology of the Cell (Garland Science).

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

Amir, E.D., Davis, K.L., Tadmor, M.D., Simonds, E.F., Levine, J.H., Bendall, S.C., Shenfeld, D.K., Krishnaswamy, S., Nolan, G.P., and Pe'er, D. (2013). viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat. Biotechnol. *31*, 545–552.

Amit, I., Regev, A., and Hacohen, N. (2011). Strategies to discover regulatory circuits of the mammalian immune system. Nat. Rev. Immunol. *11*, 873–880.

Ansel, K.M., Djuretic, I., Tanasa, B., and Rao, A. (2006). Regulation of Th2 differentiation and Il4 locus accessibility. Annu. Rev. Immunol. *24*, 607–656.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., et al. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature *504*, 451–455.

Ascenzi, P., Bocedi, A., and Marino, M. (2006). Structure-function relationship of estrogen receptor alpha and beta: impact on human health. Mol. Aspects Med. *27*, 299–402.

Aujla, S.J., Chan, Y.R., Zheng, M., Fei, M., Askew, D.J., Pociask, D.A., Reinhart, T.A., McAllister, F., Edeal, J., Gaus, K., et al. (2008). IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat. Med. *14*, 275–281.

Bachy, V., Williams, D.J., and Ibrahim, M. a. A. (2008). Altered dendritic cell function in normal pregnancy. J. Reprod. Immunol. *78*, 11–21.

Bacon, C.M., McVicar, D.W., Ortaldo, J.R., Rees, R.C., O'Shea, J.J., and Johnston, J.A. (1995a). Interleukin 12 (IL-12) induces tyrosine phosphorylation of JAK2 and TYK2: differential use of Janus family tyrosine kinases by IL-2 and IL-12. J. Exp. Med. *181*, 399–404.

Bacon, C.M., Petricoin, E.F., Ortaldo, J.R., Rees, R.C., Larner, A.C., Johnston, J.A., and O'Shea, J.J. (1995b). Interleukin 12 induces tyrosine

phosphorylation and activation of STAT4 in human lymphocytes. Proc. Natl. Acad. Sci. *92*, 7307–7311.

Banchereau, J., and Steinman, R.M. (1998). Dendritic cells and the control of immunity. Nature *392*, 245–252.

Bandyopadhyay, S., Soto-Nieves, N., and Macián, F. (2007). Transcriptional regulation of T cell tolerance. Semin. Immunol. *19*, 180–187.

Baroukh, C., Jenkins, S.L., Dannenfelser, R., and Ma'ayan, A. (2011). Genes2WordCloud: a quick way to identify biological themes from gene lists and free text. Source Code Biol. Med. *6*, 15.

Barrangou, R., and Marraffini, L.A. (2014). CRISPR-Cas systems: prokaryotes upgrade to adaptive immunity. Mol. Cell *54*, 234–244.

Baumgart, D.C., and Sandborn, W.J. (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. The Lancet *369*, 1641–1657.

Beach, D., Gonen, R., Bogin, Y., Reischl, I.G., and Yablonski, D. (2007). Dual role of SLP-76 in mediating T cell receptor-induced activation of phospholipase C-gamma1. J. Biol. Chem. *282*, 2937–2946.

Bendall, S.C., Simonds, E.F., Qiu, P., Amir, E.D., Krutzik, P.O., Finck, R., Bruggner, R.V., Melamed, R., Trejo, A., Ornatsky, O.I., et al. (2011). Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science *332*, 687–696.

Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L., Kelly, T.E., Saulsbury, F.T., Chance, P.F., and Ochs, H.D. (2001). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. *27*, 20–21.

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

Berod, L., Friedrich, C., Nandan, A., Freitag, J., Hagemann, S., Harmrolfs, K., Sandouk, A., Hesse, C., Castro, C.N., Bähre, H., et al. (2014). De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nat. Med. *20*, 1327–1333.

Berry, M., Metzger, D., and Chambon, P. (1990). Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. EMBO J. *9*, 2811–2818.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Beyer, M., Thabet, Y., Müller, R.-U., Sadlon, T., Classen, S., Lahl, K., Basu, S., Zhou, X., Bailey-Bucktrout, S.L., Krebs, W., et al. (2011). Repression of the genome organizer SATB1 in regulatory T cells is required for suppressive function and inhibition of effector differentiation. Nat. Immunol. *12*, 898–907.

Bhaumik, S., and Basu, R. (2017). Cellular and Molecular Dynamics of Th17 Differentiation and its Developmental Plasticity in the Intestinal Immune Response. Front. Immunol. *8*, 254.

Björnström, L., and Sjöberg, M. (2005). Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol. Endocrinol. Baltim. Md *19*, 833–842.

Blanco, P., Ueno, H., and Schmitt, N. (2016). T follicular helper (Tfh) cells in lupus: Activation and involvement in SLE pathogenesis. Eur. J. Immunol. *46*, 281–290.

Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P., and Gronemeyer, H. (1989). The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. Nucleic Acids Res. *17*, 2581–2595.

Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., and Förster, R. (2000). Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med. *192*, 1545–1552.

Breker, M., and Schuldiner, M. (2014). The emergence of proteome-wide technologies: systematic analysis of proteins comes of age. Nat. Rev. Mol. Cell Biol. *15*, 453–464.

Buchmann, K. (2014). Evolution of Innate Immunity: Clues from Invertebrates via Fish to Mammals. Front. Immunol. *5*.

Buck, M.D., O'Sullivan, D., and Pearce, E.L. (2015). T cell metabolism drives immunity. J. Exp. Med. *212*, 1345–1360.

Burchill, M.A., Yang, J., Vogtenhuber, C., Blazar, B.R., and Farrar, M.A. (2007). IL-2 Receptor β-Dependent STAT5 Activation Is Required for the Development of Foxp3+ Regulatory T Cells. J. Immunol. *178*, 280–290.

Bynoté, K.K., Hackenberg, J.M., Korach, K.S., Lubahn, D.B., Lane, P.H., and Gould, K.A. (2008). Estrogen receptor-alpha deficiency attenuates autoimmune disease in (NZB x NZW)F1 mice. Genes Immun. *9*, 137–152.

Chang, C.-H., Curtis, J.D., Maggi, L.B., Faubert, B., Villarino, A.V., O'Sullivan, D., Huang, S.C.-C., van der Windt, G.J.W., Blagih, J., Qiu, J., et al. (2013). Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. Cell *153*, 1239–1251.

Chang, J.T., Shevach, E.M., and Segal, B.M. (1999). Regulation of Interleukin (IL)-12 Receptor β2 Subunit Expression by Endogenous IL-12: A Critical Step in the Differentiation of Pathogenic Autoreactive T Cells. J. Exp. Med. *189*, 969–978.

Chen, J.Q., Delannoy, M., Cooke, C., and Yager, J.D. (2004). Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. Am. J. Physiol. Endocrinol. Metab. *286*, E1011-1022.

Chen, Q., Kim, Y.C., Laurence, A., Punkosdy, G.A., and Shevach, E.M. (2011). IL-2 controls the stability of Foxp3 expression in TGF-beta-induced Foxp3+ T cells in vivo. J. Immunol. Baltim. Md 1950 *186*, 6329–6337.

Chen, W., Jin, W., Tian, H., Sicurello, P., Frank, M., Orenstein, J.M., and Wahl, S.M. (2001). Requirement for transforming growth factor beta1 in controlling T cell apoptosis. J. Exp. Med. *194*, 439–453.

Chen, W., Jin, W., Hardegen, N., Lei, K.-J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J. Exp. Med. *198*, 1875–1886.

Chen, Z., Laurence, A., Kanno, Y., Pacher-Zavisin, M., Zhu, B.-M., Tato, C., Yoshimura, A., Hennighausen, L., and O'Shea, J.J. (2006). Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc. Natl. Acad. Sci. *103*, 8137–8142.

Cheng, F., Shen, Y., Mohanasundaram, P., Lindström, M., Ivaska, J., Ny, T., and Eriksson, J.E. (2016). Vimentin coordinates fibroblast proliferation and keratinocyte differentiation in wound healing via TGF-β-Slug signaling. Proc. Natl. Acad. Sci. U. S. A. *113*, E4320-4327.

Cho, J.S., Pietras, E.M., Garcia, N.C., Ramos, R.I., Farzam, D.M., Monroe, H.R., Magorien, J.E., Blauvelt, A., Kolls, J.K., Cheung, A.L., et al. (2010). IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J. Clin. Invest. *120*, 1762–1773.

Choi, Y.S., Kageyama, R., Eto, D., Escobar, T.C., Johnston, R.J., Monticelli, L., Lao, C., and Crotty, S. (2011). ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity *34*, 932–946.

Choudhary, C., and Mann, M. (2010). Decoding signalling networks by mass spectrometry-based proteomics. Nat. Rev. Mol. Cell Biol. *11*, 427–439.

Coffman, R.L., Ohara, J., Bond, M.W., Carty, J., Zlotnik, A., and Paul, W.E. (1986). B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. J. Immunol. Baltim. Md 1950 *136*, 4538–4541.

Colonna, M. (2018). Innate Lymphoid Cells: Diversity, Plasticity, and Unique Functions in Immunity. Immunity *48*, 1104–1117.

Cooper, H.S., Murthy, S.N., Shah, R.S., and Sedergran, D.J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine

colitis. Lab. Investig. J. Tech. Methods Pathol. *69*, 238–249.

Corthay, A. (2006). A three-cell model for activation of naïve T helper cells. Scand. J. Immunol. *64*, 93–96.

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

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., et al. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature *421*, 744–748.

Curtsinger, J.M., and Mescher, M.F. (2010). Inflammatory Cytokines as a Third Signal for T Cell Activation. Curr. Opin. Immunol. *22*, 333–340.

Curtsinger, J.M., Schmidt, C.S., Mondino, A., Lins, D.C., Kedl, R.M., Jenkins, M.K., and Mescher, M.F. (1999). Inflammatory Cytokines Provide a Third Signal for Activation of Naive CD4+ and CD8+ T Cells. J. Immunol. *162*, 3256–3262.

Dahlman-Wright, K., Cavailles, V., Fuqua, S.A., Jordan, V.C., Katzenellenbogen, J.A., Korach, K.S., Maggi, A., Muramatsu, M., Parker, M.G., and Gustafsson, J.-A. (2006). International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacol. Rev. *58*, 773–781.

Dai, R., Phillips, R.A., Zhang, Y., Khan, D., Crasta, O., and Ahmed, S.A. (2008). Suppression of LPS-induced Interferon-gamma and nitric oxide in splenic lymphocytes by select estrogen-regulated microRNAs: a novel mechanism of immune modulation. Blood *112*, 4591–4597.

Dang, C.V. (2012). Links between metabolism and cancer. Genes Dev. *26*, 877–890.

Delgoffe, G.M., Kole, T.P., Zheng, Y., Zarek, P.E., Matthews, K.L., Xiao, B., Worley, P.F., Kozma, S.C., and Powell, J.D. (2009). The mTOR Kinase Differentially Regulates Effector and Regulatory T Cell Lineage Commitment. Immunity *30*, 832–844.

Delgoffe, G.M., Pollizzi, K.N., Waickman, A.T., Heikamp, E., Meyers, D.J., Horton, M.R., Xiao, B., Worley, P.F., and Powell, J.D. (2011). 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.

Deng, Y., Hahn, B.H., and Tsao, B.P. (2013). Chapter 77 - Systemic Lupus Erythematosus. In Emery and Rimoin's Principles and Practice of Medical Genetics (Sixth Edition), (Oxford: Academic Press), pp. 1–22.

Dieleman, L.A., Ridwan, B.U., Tennyson, G.S., Beagley, K.W., Bucy, R.P., and Elson, C.O. (1994). Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology *107*, 1643–1652.

Dolmetsch, R.E., Lewis, R.S., Goodnow, C.C., and Healy, J.I. (1997). Differential activation of transcription factors induced by Ca2+ response amplitude and duration. Nature *386*, 855–858.

Drehmer, M.N., Andrade, D., Pereira, I.A., Marrero, A.R., Muniz, Y.C.N., de Souza, I.R., and Löfgren, S.E. (2017). Estrogen receptor alpha gene (ESR1) polymorphism can contribute to clinical findings in systemic lupus erythematosus patients. Lupus *26*, 294–298.

DuPage, M., and Bluestone, J.A. (2016). Harnessing the plasticity of $CD4^+$ T cells to treat immune-mediated disease. Nat. Rev. Immunol. *16*, 149–163.

Durant, L., Watford, W.T., Ramos, H.L., Laurence, A., Vahedi, G., Wei, L., Takahashi, H., Sun, H.-W., Kanno, Y., Powrie, F., et al. (2010). Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity *32*, 605–615.

Elo, L.L., Järvenpää, H., Tuomela, S., Raghav, S., Ahlfors, H., Laurila, K., Gupta, B., Lund, R.J., Tahvanainen, J., Hawkins, R.D., et al. (2010). Genome-wide Profiling of Interleukin-4 and STAT6 Transcription Factor Regulation of Human Th2 Cell Programming. Immunity *32*, 852–862.

Elson, C.O., Cong, Y., McCracken, V.J., Dimmitt, R.A., Lorenz, R.G., and Weaver, C.T. (2005). Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory

mechanisms of host dialogue with the microbiota. Immunol. Rev. *206*, 260–276.

Eriksson, J.E., Dechat, T., Grin, B., Helfand, B., Mendez, M., Pallari, H.-M., and Goldman, R.D. (2009). Introducing intermediate filaments: from discovery to disease. J. Clin. Invest. *119*, 1763–1771.

Ferguson, C.B., Mahsud-Dornan, S., and Patterson, R.N. (2008). Inflammatory bowel disease in pregnancy. BMJ *337*, a427–a427.

Flouriot, G., Brand, H., Denger, S., Metivier, R., Kos, M., Reid, G., Sonntag-Buck, V., and Gannon, F. (2000). Identification of a new isoform of the human estrogen receptor-alpha (hER-alpha) that is encoded by distinct transcripts and that is able to repress hER-alpha activation function 1. EMBO J. *19*, 4688–4700.

Folomeev, M., Dougados, M., Beaune, J., Kouyoumdjian, J.C., Nahoul, K., Amor, B., and Alekberova, Z. (1992). Plasma sex hormones and aromatase activity in tissues of patients with systemic lupus erythematosus. Lupus *1*, 191–195.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. *4*, 330–336.

Foss, E.J., Radulovic, D., Shaffer, S.A., Goodlett, D.R., Kruglyak, L., and Bedalov, A. (2011). Genetic Variation Shapes Protein Networks Mainly through Non-transcriptional Mechanisms. PLOS Biol. *9*, e1001144.

Frohman, E.M., Racke, M.K., and Raine, C.S. (2006). Multiple sclerosis--the plaque and its pathogenesis. N. Engl. J. Med. *354*, 942–955.

Galien, R., and Garcia, T. (1997). Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site. Nucleic Acids Res. *25*, 2424–2429.

Gatson, N.N., Williams, J.L., Powell, N.D., McClain, M.A., Hennon, T.R., Robbins, P.D., and Whitacre, C.C. (2011). Induction of pregnancy during established EAE halts progression of CNS autoimmune injury via pregnancy-specific serum factors. J. Neuroimmunol. *230*, 105–113.

Ghazalpour, A., Bennett, B., Petyuk, V.A., Orozco, L., Hagopian, R., Mungrue, I.N., Farber, C.R., Sinsheimer, J., Kang, H.M., Furlotte, N., et al. (2011). Comparative Analysis of Proteome and Transcriptome Variation in Mouse. PLOS Genet. *7*, e1001393.

Ghoreschi, K., Laurence, A., Yang, X.-P., Tato, C.M., McGeachy, M.J., Konkel, J., Ramos, H.L., Wei, L., Davidson, T., Bouladoux, N., et al. (2010). Generation of Pathogenic Th17 Cells in the Absence of TGF-β Signaling. Nature *467*, 967–971.

Godet, P.G., May, G.R., and Sutherland, L.R. (1995). Meta-analysis of the role of oral contraceptive agents in inflammatory bowel disease. Gut *37*, 668–673.

Goll, M.G., and Bestor, T.H. (2005). Eukaryotic cytosine methyltransferases. Annu. Rev. Biochem. *74*, 481–514.

Gorelik, L., Fields, P.E., and Flavell, R.A. (2000). Cutting edge: TGF-beta inhibits Th type 2 development through inhibition of GATA-3 expression. J. Immunol. Baltim. Md 1950 *165*, 4773–4777.

Gorelik, L., Constant, S., and Flavell, R.A. (2002). Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. J. Exp. Med. *195*, 1499–1505.

Graessel, A., Hauck, S.M., von Toerne, C., Kloppmann, E., Goldberg, T., Koppensteiner, H., Schindler, M., Knapp, B., Krause, L., Dietz, K., et al. (2015). A Combined Omics Approach to Generate the Surface Atlas of Human Naive CD4+ T Cells during Early T-Cell Receptor Activation. Mol. Cell. Proteomics MCP *14*, 2085–2102.

Green, S., Walter, P., Kumar, V., Krust, A., Bornert, J.M., Argos, P., and Chambon, P. (1986). Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature *320*, 134–139.

Greenberg, S., and Grinstein, S. (2002). Phagocytosis and innate immunity. Curr. Opin. Immunol. *14*, 136–145.

Guppy, M., Greiner, E., and Brand, K. (1993). The role of the Crabtree effect and an endogenous fuel in the energy metabolism of resting and

proliferating thymocytes. Eur. J. Biochem. *212*, 95–99.

Gutcher, I., Donkor, M.K., Ma, Q., Rudensky, A.Y., Flavell, R.A., and Li, M.O. (2011). Autocrine transforming growth factor-β1 promotes in vivo Th17 cell differentiation. Immunity *34*, 396–408.

Harnish, D.C., Albert, L.M., Leathurby, Y., Eckert, A.M., Ciarletta, A., Kasaian, M., and Keith, J.C. (2004). Beneficial effects of estrogen treatment in the HLA-B27 transgenic rat model of inflammatory bowel disease. Am. J. Physiol.-Gastrointest. Liver Physiol. *286*, G118–G125.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. *6*, 1123–1132.

Haruki, Y., Seiki, K., Enomoto, T., Fujii, H., and Sakabe, K. (1983). Estrogen receptor in the "non-lymphocytes" in the thymus of the ovariectomized rat. Tokai J. Exp. Clin. Med. *8*, 31–39.

Haxhinasto, S., Mathis, D., and Benoist, C. (2008). The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J. Exp. Med. *205*, 565–574.

Hayashi, K., and Altman, A. (2007). Protein kinase C theta (PKCtheta): a key player in T cell life and death. Pharmacol. Res. *55*, 537–544.

Hendrix, M.J.C., Seftor, E.A., Chu, Y.-W., Trevor, K.T., and Seftor, R.E.B. (1996). Role of intermediate filaments in migration, invasion and metastasis. Cancer Metastasis Rev. *15*, 507–525.

Heng, T.S.P., Painter, M.W., Consortium, T.I.G.P., Elpek, K., Lukacs-Kornek, V., Mauermann, N., Turley, S.J., Koller, D., Kim, F.S., Wagers, A.J., et al. (2008). The Immunological Genome Project: networks of gene expression in immune cells.

Hillenmeyer, M.E., Fung, E., Wildenhain, J., Pierce, S.E., Hoon, S., Lee, W., Proctor, M., St Onge, R.P., Tyers, M., Koller, D., et al. (2008). The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science *320*, 362–365.

Holland, S.M., DeLeo, F.R., Elloumi, H.Z., Hsu, A.P., Uzel, G., Brodsky, N., Freeman, A.F., Demidowich, A., Davis, J., Turner, M.L., et al. (2007). STAT3 mutations in the hyper-IgE syndrome. N. Engl. J. Med. *357*, 1608–1619.

Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012). Interactions between the microbiota and the immune system. Science *336*, 1268–1273.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of Regulatory T Cell Development by the Transcription Factor Foxp3. Science *299*, 1057–1061.

Hornbeck, P.V., Garrels, J.I., Capetanaki, Y., and Heimer, S. (1993). Vimentin expression is differentially regulated by IL-2 and IL-4 in murine T cells. J. Immunol. Baltim. Md 1950 *151*, 4013–4021.

Horwitz, D.A., Zheng, S.G., Wang, J., and Gray, J.D. (2008). Critical role of IL-2 and TGF-beta in generation, function and stabilization of Foxp3+CD4+ Treg. Eur. J. Immunol. *38*, 912–915.

Hosp, F., and Mann, M. (2017). A Primer on Concepts and Applications of Proteomics in Neuroscience. Neuron *96*, 558–571.

Hsieh, C.-H., Nickel, E.A., Chen, J., Schwacha, M.G., Choudhry, M.A., Bland, K.I., and Chaudry, I.H. (2009). Mechanism of the salutary effects of estrogen on kupffer cell phagocytic capacity following trauma-hemorrhage: pivotal role of Akt activation. J. Immunol. Baltim. Md 1950 *182*, 4406–4414.

Hsieh, C.S., Macatonia, S.E., Tripp, C.S., Wolf, S.F., O'Garra, A., and Murphy, K.M. (1993). Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science *260*, 547–549.

Hu, A., Noble, W.S., and Wolf-Yadlin, A. (2016). Technical advances in proteomics: new developments in data-independent acquisition. F1000Research *5*.

Ichiyama, K., Chen, T., Wang, X., Yan, X., Kim, B.-S., Tanaka, S., Ndiaye-Lobry, D., Deng, Y.,

Zou, Y., Zheng, P., et al. (2015). The methylcytosine dioxygenase Tet2 promotes DNA demethylation and activation of cytokine gene expression in T cells. Immunity *42*, 613–626.

Iguchi-Ariga, S.M., and Schaffner, W. (1989). CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. Genes Dev. *3*, 612–619.

Inui, A., Ogasawara, H., Naito, T., Sekigawa, I., Takasaki, Y., Hayashida, Y., Takamori, K., and Ogawa, H. (2007). Estrogen receptor expression by peripheral blood mononuclear cells of patients with systemic lupus erythematosus. Clin. Rheumatol. *26*, 1675–1678.

Ishigame, H., Kakuta, S., Nagai, T., Kadoki, M., Nambu, A., Komiyama, Y., Fujikado, N., Tanahashi, Y., Akitsu, A., Kotaki, H., et al. (2009). Differential Roles of Interleukin-17A and -17F in Host Defense against Mucoepithelial Bacterial Infection and Allergic Responses. Immunity *30*, 108–119.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell *126*, 1121–1133.

Ivanov, I.I., Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Jacobson, N.G., Szabo, S.J., Weber-Nordt, R.M., Zhong, Z., Schreiber, R.D., Darnell, J.E., and Murphy, K.M. (1995). Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (Stat)3 and Stat4. J. Exp. Med. *181*, 1755–1762.

Janeway, C.A.J., Travers, P., Walport, M., Shlomchik, M.J., Jr, C.A.J., Travers, P., Walport, M., and Shlomchik, M.J. (2001). Immunobiology (Garland Science).

Jia, M., Dahlman-Wright, K., and Gustafsson, J.-Å. (2015). Estrogen receptor alpha and beta in health and disease. Best Pract. Res. Clin. Endocrinol. Metab. *29*, 557–568.

Johansson, M., Arlestig, L., Möller, B., Smedby, T., and Rantapää-Dahlqvist, S. (2005). Oestrogen receptor {alpha} gene polymorphisms in systemic lupus erythematosus. Ann. Rheum. Dis. *64*, 1611–1617.

Johnston, R.J., Poholek, A.C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., Dent, A.L., Craft, J., and Crotty, S. (2009). Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science *325*, 1006–1010.

Jones, P.A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat. Rev. Genet. *13*, 484–492.

Jones, R.G., and Pearce, E.J. (2017). MenTORing Immunity: mTOR Signaling in the Development and Function of Tissue-Resident Immune Cells. Immunity *46*, 730–742.

Josefowicz, S.Z., Wilson, C.B., and Rudensky, A.Y. (2009). Cutting Edge: TCR Stimulation Is Sufficient for Induction of Foxp3 Expression in the Absence of DNA Methyltransferase 1. J. Immunol. *182*, 6648–6652.

Justice, M.J., Siracusa, L.D., Gilbert, D.J., Heisterkamp, N., Groffen, J., Chada, K., Silan, C.M., Copeland, N.G., and Jenkins, N.A. (1990). A genetic linkage map of mouse chromosome 10: localization of eighteen molecular markers using a single interspecific backcross. Genetics *125*, 855–866.

Kanda, N., and Tamaki, K. (1999). Estrogen enhances immunoglobulin production by human PBMCs. J. Allergy Clin. Immunol. *103*, 282–288.

Kane, S.V., and Reddy, D. (2008). Hormonal Replacement Therapy After Menopause Is Protective of Disease Activity in Women With Inflammatory Bowel Disease. Am. J. Gastroenterol. *103*, 1193–1196.

Kang, K.Y., Kim, Y.-K., Yi, H., Kim, J., Jung, H.-R., Kim, I.J., Cho, J.-H., Park, S.-H., Kim, H.-Y., and Ju, J.H. (2013). Metformin downregulates Th17 cells differentiation and

attenuates murine autoimmune arthritis. Int. Immunopharmacol. *16*, 85–92.

Kaplan, M.H., Sun, Y.L., Hoey, T., and Grusby, M.J. (1996a). Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature *382*, 174–177.

Kaplan, M.H., Schindler, U., Smiley, S.T., and Grusby, M.J. (1996b). Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity *4*, 313–319.

Karpuzoglu, E., Phillips, R.A., Dai, R., Graniello, C., Gogal, R.M., and Ahmed, S.A. (2009). Signal transducer and activation of transcription (STAT) 4beta, a shorter isoform of interleukin-12-induced STAT4, is preferentially activated by estrogen. Endocrinology *150*, 1310–1320.

Kassi, E., and Moutsatsou, P. (2010). Estrogen Receptor Signaling and Its Relationship to Cytokines in Systemic Lupus Erythematosus. J. Biomed. Biotechnol. *2010*.

Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., et al. (1995). Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science *270*, 1491–1494.

Kerfoot, S.M., Yaari, G., Patel, J.R., Johnson, K.L., Gonzalez, D.G., Kleinstein, S.H., and Haberman, A.M. (2011). Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity *34*, 947–960.

Khalili, H., Higuchi, L.M., Ananthakrishnan, A.N., Richter, J.M., Feskanich, D., Fuchs, C.C., and Chan, A.T. (2013). Oral contraceptives, reproductive factors and risk of inflammatory bowel disease. Gut *62*, 1153–1159.

Khattri, R., Cox, T., Yasayko, S.-A., and Ramsdell, F. (2003). An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat. Immunol. *4*, 337–342.

Kidd, M.E., Shumaker, D.K., and Ridge, K.M. (2014). The Role of Vimentin Intermediate Filaments in the Progression of Lung Cancer. Am. J. Respir. Cell Mol. Biol. *50*, 1–6.

Kiesler, P., Fuss, I.J., and Strober, W. (2015). Experimental Models of Inflammatory Bowel Diseases. Cell. Mol. Gastroenterol. Hepatol. *1*, 154–170.

Kim, H.-P., and Leonard, W.J. (2007). CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. J. Exp. Med. *204*, 1543–1551.

Kim, C.H., Rott, L.S., Clark-Lewis, I., Campbell, D.J., Wu, L., and Butcher, E.C. (2001). Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. J. Exp. Med. *193*, 1373–1381.

Kitano, M., Moriyama, S., Ando, Y., Hikida, M., Mori, Y., Kurosaki, T., and Okada, T. (2011). Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. Immunity *34*, 961–972.

Klein-Hitpass, L., Ryffel, G.U., Heitlinger, E., and Cato, A.C. (1988). A 13 bp palindrome is a functional estrogen responsive element and interacts specifically with estrogen receptor. Nucleic Acids Res. *16*, 647–663.

Klinge, C.M. (2001). Estrogen receptor interaction with estrogen response elements. Nucleic Acids Res. *29*, 2905–2919.

Kokkinos, M.I., Wafai, R., Wong, M.K., Newgreen, D.F., Thompson, E.W., and Waltham, M. (2007). Vimentin and epithelial-mesenchymal transition in human breast cancer--observations in vitro and in vivo. Cells Tissues Organs *185*, 191–203.

Komatsu, N., Mariotti-Ferrandiz, M.E., Wang, Y., Malissen, B., Waldmann, H., and Hori, S. (2009). Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proc. Natl. Acad. Sci. U. S. A. *106*, 1903–1908.

Komatsu, N., Okamoto, K., Sawa, S., Nakashima, T., Oh-Hora, M., Kodama, T., Tanaka, S., Bluestone, J.A., and Takayanagi, H. (2014). Pathogenic conversion of Foxp3(+) T cells into TH17 cells in autoimmune arthritis. Nat. Med. *20*, 62–68.

Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., and Köhler, G. (1993). Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature *362*, 245–248.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell *128*, 693–705.

Krieglstein, C.F., Cerwinka, W.H., Sprague, A.G., Laroux, F.S., Grisham, M.B., Koteliansky, V.E., Senninger, N., Granger, D.N., and de Fougerolles, A.R. (2002). Collagen-binding integrin alpha1beta1 regulates intestinal inflammation in experimental colitis. J. Clin. Invest. *110*, 1773–1782.

Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., Ferreira, G., Iyer, N., LaRusch, J., Pak, B., Taghavi, P., et al. (2003). Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res. *63*, 1138–1143.

Kurebayashi, Y., Nagai, S., Ikejiri, A., Ohtani, M., Ichiyama, K., Baba, Y., Yamada, T., Egami, S., Hoshii, T., Hirao, A., et al. (2012). PI3K-Akt-mTORC1-S6K1/2 Axis Controls Th17 Differentiation by Regulating Gfi1 Expression and Nuclear Translocation of RORγ. Cell Rep. *1*, 360–373.

Ladle, B.H., Li, K.-P., Phillips, M.J., Pucsek, A.B., Haile, A., Powell, J.D., Jaffee, E.M., Hildeman, D.A., and Gamper, C.J. (2016). De novo DNA methylation by DNA methyltransferase 3a controls early effector CD8+ T-cell fate decisions following activation. Proc. Natl. Acad. Sci. U. S. A. *113*, 10631–10636.

Lahita, R.G., Bradlow, L., Fishman, J., and Kunkel, H.G. (1982). Estrogen metabolism in systemic lupus erythematosus: patients and family members. Arthritis Rheum. *25*, 843–846.

Lal, G., Zhang, N., van der Touw, W., Ding, Y., Ju, W., Bottinger, E.P., Reid, S.P., Levy, D.E., and Bromberg, J.S. (2009). Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J. Immunol. Baltim. Md 1950 *182*, 259–273.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. *201*, 233–240.

Laplante, M., and Sabatini, D.M. (2009). mTOR signaling at a glance. J. Cell Sci. *122*, 3589–3594.

Larance, M., and Lamond, A.I. (2015). Multidimensional proteomics for cell biology. Nat. Rev. Mol. Cell Biol. *16*, 269–280.

Laurence, A., Tato, C.M., Davidson, T.S., Kanno, Y., Chen, Z., Yao, Z., Blank, R.B., Meylan, F., Siegel, R., Hennighausen, L., et al. (2007). Interleukin-2 Signaling via STAT5 Constrains T Helper 17 Cell Generation. Immunity *26*, 371–381.

Le Gros, G., Ben-Sasson, S.Z., Seder, R., Finkelman, F.D., and Paul, W.E. (1990). Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. J. Exp. Med. *172*, 921–929.

Lee, Y., and Kuchroo, V. (2015). Defining the functional states of Th17 cells. F1000Research.

Lee, P.P., Fitzpatrick, D.R., Beard, C., Jessup, H.K., Lehar, S., Makar, K.W., Pérez-Melgosa, M., Sweetser, M.T., Schlissel, M.S., Nguyen, S., et al. (2001). A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. Immunity *15*, 763–774.

Lee, Y.J., Shin, K.S., Kang, S.W., Lee, C.K., Yoo, B., Cha, H.S., Koh, E.M., Yoon, S.J., and Lee, J. (2004). Association of the oestrogen receptor alpha gene polymorphisms with disease onset in systemic lupus erythematosus. Ann. Rheum. Dis. *63*, 1244–1249.

Lee, Y.K., Turner, H., Maynard, C.L., Oliver, J.R., Chen, D., Elson, C.O., and Weaver, C.T. (2009). Late developmental plasticity in the T helper 17 lineage. Immunity *30*, 92–107.

Lélu, K., Laffont, S., Delpy, L., Paulet, P.-E., Périnat, T., Tschanz, S.A., Pelletier, L., Engelhardt, B., and Guéry, J.-C. (2011). Estrogen receptor α signaling in T lymphocytes is required for estradiol-mediated inhibition of Th1 and Th17 cell differentiation and protection against experimental autoimmune encephalomyelitis. J. Immunol. Baltim. Md 1950 *187*, 2386–2393.

Lian, N., Lin, T., Liu, W., Wang, W., Li, L., Sun, S., Nyman, J.S., and Yang, X. (2012). Transforming Growth Factor β Suppresses Osteoblast Differentiation via the Vimentin Activating Transcription Factor 4 (ATF4) Axis. J. Biol. Chem. *287*, 35975–35984.

Lighvani, A.A., Frucht, D.M., Jankovic, D., Yamane, H., Aliberti, J., Hissong, B.D., Nguyen, B.V., Gadina, M., Sher, A., Paul, W.E., et al. (2001). T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc. Natl. Acad. Sci. U. S. A. *98*, 15137–15142.

Liu, H.Y., Buenafe, A.C., Matejuk, A., Ito, A., Zamora, A., Dwyer, J., Vandenbark, A.A., and Offner, H. (2002). Estrogen inhibition of EAE involves effects on dendritic cell function. J. Neurosci. Res. *70*, 238–248.

Liu, Y., Zhang, P., Li, J., Kulkarni, A.B., Perruche, S., and Chen, W. (2008). A critical function for TGF-beta signaling in the development of natural CD4+CD25+Foxp3+ regulatory T cells. Nat. Immunol. *9*, 632–640.

Loker, E.S., Adema, C.M., Zhang, S.-M., and Kepler, T.B. (2004). Invertebrate immune systems – not homogeneous, not simple, not well understood. Immunol. Rev. *198*, 10–24.

Lowery, J., Kuczmarski, E.R., Herrmann, H., and Goldman, R.D. (2015). Intermediate Filaments Play a Pivotal Role in Regulating Cell Architecture and Function. J. Biol. Chem. *290*, 17145–17153.

Lu, L., Lan, Q., Li, Z., Zhou, X., Gu, J., Li, Q., Wang, J., Chen, M., Liu, Y., Shen, Y., et al. (2014). Critical role of all-trans retinoic acid in stabilizing human natural regulatory T cells under inflammatory conditions. Proc. Natl. Acad. Sci. *111*, E3432–E3440.

Lu, L., Barbi, J., and Pan, F. (2017). The regulation of immune tolerance by FOXP3. Nat. Rev. Immunol. *17*, 703–717.

Ma, C.S., Chew, G.Y.J., Simpson, N., Priyadarshi, A., Wong, M., Grimbacher, B., Fulcher, D.A., Tangye, S.G., and Cook, M.C. (2008). Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. J. Exp. Med. *205*, 1551–1557.

Macintyre, A.N., Gerriets, V.A., Nichols, A.G., Michalek, R.D., Rudolph, M.C., Deoliveira, D., Anderson, S.M., Abel, E.D., Chen, B.J., Hale, L.P., et al. (2014). The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. Cell Metab. *20*, 61–72.

MacIver, N.J., Michalek, R.D., and Rathmell, J.C. (2013). Metabolic regulation of T lymphocytes. Annu. Rev. Immunol. *31*, 259–283.

Mader, S., Kumar, V., de Verneuil, H., and Chambon, P. (1989). Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element. Nature *338*, 271–274.

Maier, T., Güell, M., and Serrano, L. (2009). Correlation of mRNA and protein in complex biological samples. FEBS Lett. *583*, 3966–3973.

Manel, N., Unutmaz, D., and Littman, D.R. (2008). The differentiation of human TH-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORγT. Nat. Immunol. *9*, 641–649.

Mangelsdorf, D.J., Thummel, C., Beato, M., Herrlich, P., Schütz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., et al. (1995). The nuclear receptor superfamily: the second decade. Cell *83*, 835–839.

Marino, M., Galluzzo, P., and Ascenzi, P. (2006). Estrogen Signaling Multiple Pathways to Impact Gene Transcription. Curr. Genomics *7*, 497–508.

Marraffini, L.A., and Sontheimer, E.J. (2010). Self versus non-self discrimination during CRISPR RNA-directed immunity. Nature *463*, 568–571.

Mathur, A.N., Chang, H.-C., Zisoulis, D.G., Stritesky, G.L., Yu, Q., O'Malley, J.T., Kapur, R., Levy, D.E., Kansas, G.S., and Kaplan, M.H. (2007). Stat3 and Stat4 direct development of IL-17-secreting Th cells. J. Immunol. Baltim. Md 1950 *178*, 4901–4907.

McInerney, E.M., and Katzenellenbogen, B.S. (1996). Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. J. Biol. Chem. *271*, 24172–24178.

McKinney, E.F., and Smith, K.G.C. (2018). Metabolic exhaustion in infection, cancer and autoimmunity. Nat. Immunol. *19*, 213–221.

Merle, N.S., Noe, R., Halbwachs-Mecarelli, L., Fremeaux-Bacchi, V., and Roumenina, L.T. (2015). Complement System Part II: Role in Immunity. Front. Immunol. *6*.

Mestas, J., and Hughes, C.C.W. (2004). Of mice and not men: differences between mouse and human immunology. J. Immunol. Baltim. Md 1950 *172*, 2731–2738.

Meyer, M.E., Gronemeyer, H., Turcotte, B., Bocquel, M.T., Tasset, D., and Chambon, P. (1989). Steroid hormone receptors compete for factors that mediate their enhancer function. Cell *57*, 433–442.

Michalek, R.D., Gerriets, V.A., Jacobs, S.R., Macintyre, A.N., MacIver, N.J., Mason, E.F., Sullivan, S.A., Nichols, A.G., and Rathmell, J.C. (2011). 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*, 3299–3303.

Mikami, Y., Kanai, T., Sujino, T., Ono, Y., Hayashi, A., Okazawa, A., Kamada, N., Matsuoka, K., Hisamatsu, T., Okamoto, S., et al. (2010). Competition between colitogenic Th1 and Th17 cells contributes to the amelioration of colitis. Eur. J. Immunol. *40*, 2409–2422.

Monks, C.R.F., Freiberg, B.A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. Nature *395*, 82–86.

Mor-Vaknin, N., Punturieri, A., Sitwala, K., and Markovitz, D.M. (2003). Vimentin is secreted by activated macrophages. Nat. Cell Biol. *5*, 59–63.

Mor-Vaknin, N., Legendre, M., Yu, Y., Serezani, C.H.C., Garg, S.K., Jatzek, A., Swanson, M.D., Gonzalez-Hernandez, M.J., Teitz-Tennenbaum, S., Punturieri, A., et al. (2013). Murine colitis is mediated by vimentin. Sci. Rep. *3*, 1045.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. *136*, 2348–2357.

Moss, S.B., and Gerton, G.L. (2001). A-kinase anchor proteins in endocrine systems and reproduction. Trends Endocrinol. Metab. TEM *12*, 434–440.

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., and Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science *317*, 256–260.

Mucida, D., Pino-Lagos, K., Kim, G., Nowak, E., Benson, M.J., Kronenberg, M., Noelle, R.J., and Cheroutre, H. (2009). Retinoic acid can directly promote TGF-beta-mediated Foxp3(+) Treg cell conversion of naive T cells. Immunity *30*, 471–472; author reply 472-473.

Mukasa, R., Balasubramani, A., Lee, Y.K., Whitley, S.K., Weaver, B.T., Shibata, Y., Crawford, G.E., Hatton, R.D., and Weaver, C.T. (2010). Epigenetic instability of cytokine and transcription factor gene loci underlies plasticity of the T helper 17 cell lineage. Immunity *32*, 616–627.

Nakayama, T., and Yamashita, M. (2010). The TCR-mediated signaling pathways that control the direction of helper T cell differentiation. Semin. Immunol. *22*, 303–309.

Nieminen, M., Henttinen, T., Merinen, M., Marttila-Ichihara, F., Eriksson, J.E., and Jalkanen, S. (2006). Vimentin function in lymphocyte adhesion and transcellular migration. Nat. Cell Biol. *8*, 156–162.

Nilsson, S., Mäkelä, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M., and Gustafsson, J.-Å. (2001). Mechanisms of estrogen action. Physiol. Rev. *81*, 1535–1565.

Nurieva, R.I., Chung, Y., Martinez, G.J., Yang, X.O., Tanaka, S., Matskevitch, T.D., Wang, Y.-H., and Dong, C. (2009). Bcl6 mediates the development of T follicular helper cells. Science *325*, 1001–1005.

Offner, H., and Polanczyk, M. (2006). A potential role for estrogen in experimental autoimmune encephalomyelitis and multiple sclerosis. Ann. N. Y. Acad. Sci. *1089*, 343–372.

Okayasu, I., Hatakeyama, S., Yamada, M., Ohkusa, T., Inagaki, Y., and Nakaya, R. (1990).

A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology *98*, 694–702.

Onodera, A., Yamashita, M., Endo, Y., Kuwahara, M., Tofukuji, S., Hosokawa, H., Kanai, A., Suzuki, Y., and Nakayama, T. (2010). STAT6-mediated displacement of polycomb by trithorax complex establishes long-term maintenance of GATA3 expression in T helper type 2 cells. J. Exp. Med. *207*, 2493–2506.

Oppmann, B., Lesley, R., Blom, B., Timans, J.C., Xu, Y., Hunte, B., Vega, F., Yu, N., Wang, J., Singh, K., et al. (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity *13*, 715–725.

Ostanin, D.V., Bao, J., Koboziev, I., Gray, L., Robinson-Jackson, S.A., Kosloski-Davidson, M., Price, V.H., and Grisham, M.B. (2009). T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am. J. Physiol. - Gastrointest. Liver Physiol. *296*, G135–G146.

Ouyang, W., Beckett, O., Ma, Q., and Li, M.O. (2010). Transforming growth factor-beta signaling curbs thymic negative selection promoting regulatory T cell development. Immunity *32*, 642–653.

Pagès, F., Ragueneau, M., Rottapel, R., Truneh, A., Nunes, J., Imbert, J., and Olive, D. (1994). Binding of phosphatidylinositol-3-OH kinase to CD28 is required for T-cell signalling. Nature *369*, 327–329.

Pardoll, D.M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer *12*, 252–264.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.-H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., et al. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. *6*, 1133–1141.

Pastor, W.A., Aravind, L., and Rao, A. (2013). TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat. Rev. Mol. Cell Biol. *14*, 341–356.

Pennington, K.L., Chan, T.Y., Torres, M.P., and Andersen, J.L. (2018). The dynamic and stress-adaptive signaling hub of 14-3-3: emerging mechanisms of regulation and context-dependent protein–protein interactions. Oncogene 1.

Pettersson, A., Ciumas, C., Chirsky, V., Link, H., Huang, Y.-M., and Xiao, B.-G. (2004). Dendritic cells exposed to estrogen in vitro exhibit therapeutic effects in ongoing experimental allergic encephalomyelitis. J. Neuroimmunol. *156*, 58–65.

Pettinelli, C.B., and McFarlin, D.E. (1981). Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement for Lyt 1+ 2- T lymphocytes. J. Immunol. Baltim. Md 1950 *127*, 1420–1423.

Phiel, K.L., Henderson, R.A., Adelman, S.J., and Elloso, M.M. (2005). Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. Immunol. Lett. *97*, 107–113.

Picotti, P., and Aebersold, R. (2012). Selected reaction monitoring–based proteomics: workflows, potential, pitfalls and future directions. Nat. Methods *9*, 555–566.

Pierdominici, M., Maselli, A., Varano, B., Barbati, C., Cesaro, P., Spada, C., Zullo, A., Lorenzetti, R., Rosati, M., Rainaldi, G., et al. (2015). Linking estrogen receptor β expression with inflammatory bowel disease activity. Oncotarget *6*, 40443–40451.

Polanczyk, M., Zamora, A., Subramanian, S., Matejuk, A., Hess, D.L., Blankenhorn, E.P., Teuscher, C., Vandenbark, A.A., and Offner, H. (2003). The Protective Effect of 17β-Estradiol on Experimental Autoimmune Encephalomyelitis Is Mediated through Estrogen Receptor-α. Am. J. Pathol. *163*, 1599–1605.

Polanczyk, M.J., Carson, B.D., Subramanian, S., Afentoulis, M., Vandenbark, A.A., Ziegler, S.F., and Offner, H. (2004). Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J. Immunol. Baltim. Md 1950 *173*, 2227–2230.

Polanczyk, M.J., Hopke, C., Vandenbark, A.A., and Offner, H. (2007). Treg suppressive activity involves estrogen-dependent expression of programmed death-1 (PD-1). Int. Immunol. *19*, 337–343.

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

Ponglikitmongkol, M., Green, S., and Chambon, P. (1988). Genomic organization of the human oestrogen receptor gene. EMBO J. *7*, 3385–3388.

Popgeorgiev, N., Jabbour, L., and Gillet, G. (2018). Subcellular Localization and Dynamics of the Bcl-2 Family of Proteins. Front. Cell Dev. Biol. *6*.

Powell, J.D., and Delgoffe, G.M. (2010). The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. Immunity *33*, 301–311.

Powrie, F., Leach, M.W., Mauze, S., Caddle, L.B., and Coffman, R.L. (1993). Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int. Immunol. *5*, 1461–1471.

Powrie, F., Leach, M.W., Mauze, S., Menon, S., Caddle, L.B., and Coffman, R.L. (1994). Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. Immunity *1*, 553–562.

Quinn, L.M., and Richardson, H. (2004). Bcl-2 in Cell Cycle Regulation. Cell Cycle *3*, 6–8.

Rauniyar, N., and Yates, J.R. (2014). Isobaric Labeling-Based Relative Quantification in Shotgun Proteomics. J. Proteome Res. *13*, 5293–5309.

Ray, A., Prefontaine, K.E., and Ray, P. (1994). Down-modulation of interleukin-6 gene expression by 17 beta-estradiol in the absence of high affinity DNA binding by the estrogen receptor. J. Biol. Chem. *269*, 12940–12946.

Revankar, C.M., Cimino, D.F., Sklar, L.A., Arterburn, J.B., and Prossnitz, E.R. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science *307*, 1625–1630.

Reynolds, L.F., de Bettignies, C., Norton, T., Beeser, A., Chernoff, J., and Tybulewicz, V.L.J. (2004). Vav1 transduces T cell receptor signals to the activation of the Ras/ERK pathway via LAT, Sos, and RasGRP1. J. Biol. Chem. *279*, 18239–18246.

Ribeiro, R.C.J., Kushner, P.J., and Baxter, J.D. (1995). The nuclear hormone receptor gene superfamily. Annu. Rev. Med. *46*, 443–453.

Riis, L., Vind, I., Politi, P., Wolters, F., Vermeire, S., Tsianos, E., Freitas, J., Mouzas, I., Ochoa, V.R., O'Morain, C., et al. (2006). Does Pregnancy Change the Disease Course? A Study in a European Cohort of Patients with Inflammatory Bowel Disease. Am. J. Gastroenterol. *101*, 1539–1545.

Rongvaux, A., Willinger, T., Martinek, J., Strowig, T., Gearty, S.V., Teichmann, L.L., Saito, Y., Marches, F., Halene, S., Palucka, A.K., et al. (2014). Development and function of human innate immune cells in a humanized mouse model. Nat. Biotechnol. *32*, 364–372.

Rubtsova, K., Marrack, P., and Rubtsov, A.V. (2015). Sexual dimorphism in autoimmunity. J. Clin. Invest. *125*, 2187–2193.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. Baltim. Md 1950 *155*, 1151–1164.

Samoilova, E.B., Horton, J.L., Hilliard, B., Liu, T.-S.T., and Chen, Y. (1998). IL-6-Deficient Mice Are Resistant to Experimental Autoimmune Encephalomyelitis: Roles of IL-6 in the Activation and Differentiation of Autoreactive T Cells. J. Immunol. *161*, 6480–6486.

Samstein, R.M., Arvey, A., Josefowicz, S.Z., Peng, X., Reynolds, A., Sandstrom, R., Neph, S., Sabo, P., Kim, J.M., Liao, W., et al. (2012). Foxp3 exploits a preexistent enhancer landscape for regulatory T cell lineage specification. Cell *151*, 153–166.

dos Santos, G., Rogel, M.R., Baker, M.A., Troken, J.R., Urich, D., Morales-Nebreda, L., Sennello, J.A., Kutuzov, M.A., Sitikov, A., Davis, J.M., et al. (2015). Vimentin regulates activation of the NLRP3 inflammasome. Nat. Commun. *6*, 6574.

Satelli, A., and Li, S. (2011). Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell. Mol. Life Sci. CMLS *68*, 3033–3046.

Sauer, S., Bruno, L., Hertweck, A., Finlay, D., Leleu, M., Spivakov, M., Knight, Z.A., Cobb, B.S., Cantrell, D., O'Connor, E., et al. (2008). T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc. Natl. Acad. Sci. U. S. A. *105*, 7797–7802.

Savaryn, J.P., Toby, T.K., and Kelleher, N.L. (2016). A researcher's guide to mass spectrometry-based proteomics. PROTEOMICS *16*, 2435–2443.

Schaerli, P., Willimann, K., Lang, A.B., Lipp, M., Loetscher, P., and Moser, B. (2000). CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J. Exp. Med. *192*, 1553–1562.

Schmidt, A., Eriksson, M., Shang, M.-M., Weyd, H., and Tegnér, J. (2016). Comparative Analysis of Protocols to Induce Human CD4+Foxp3+ Regulatory T Cells by Combinations of IL-2, TGF-beta, Retinoic Acid, Rapamycin and Butyrate. PLOS ONE *11*, e0148474.

Schwandner, R., Dziarski, R., Wesche, H., Rothe, M., and Kirschning, C.J. (1999). Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J. Biol. Chem. *274*, 17406–17409.

Selmi, C., Brunetta, E., Raimondo, M.G., and Meroni, P.L. (2012). The X chromosome and the sex ratio of autoimmunity. Autoimmun. Rev. *11*, A531-537.

Seok, J., Warren, H.S., Cuenca, A.G., Mindrinos, M.N., Baker, H.V., Xu, W., Richards, D.R., McDonald-Smith, G.P., Gao, H., Hennessy, L., et al. (2013). Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc. Natl. Acad. Sci. U. S. A. *110*, 3507–3512.

Shah, D.K., and Zuniga-Pflucker, J.C. (2014). An Overview of the Intrathymic Intricacies of T Cell Development. J. Immunol. *192*, 4017–4023.

Shang, Y., Hu, X., DiRenzo, J., Lazar, M.A., and Brown, M. (2000). Cofactor Dynamics and Sufficiency in Estrogen Receptor–Regulated Transcription. Cell *103*, 843–852.

Shi, G., Cox, C.A., Vistica, B.P., Tan, C., Wawrousek, E.F., and Gery, I. (2008). Phenotype switching by inflammation-inducing polarized Th17 cells, but not by Th1 cells. J. Immunol. Baltim. Md 1950 *181*, 7205–7213.

Shi, J.-F., Li, X.-J., Si, X.-X., Li, A.-D., Ding, H.-J., Han, X., and Sun, Y.-J. (2012). ERα positively regulated DNMT1 expression by binding to the gene promoter region in human breast cancer MCF-7 cells. Biochem. Biophys. Res. Commun. *427*, 47–53.

Shi, T., Song, E., Nie, S., Rodland, K.D., Liu, T., Qian, W.-J., and Smith, R.D. (2016). Advances in targeted proteomics and applications to biomedical research. Proteomics *16*, 2160–2182.

Shim, G.-J., Gherman, D., Kim, H.-J., Omoto, Y., Iwase, H., Bouton, D., Kis, L.L., Andersson, C.T., Warner, M., and Gustafsson, J.-A. (2006). Differential expression of oestrogen receptors in human secondary lymphoid tissues. J. Pathol. *208*, 408–414.

Shimoda, K., van Deursen, J., Sangster, M.Y., Sarawar, S.R., Carson, R.T., Tripp, R.A., Chu, C., Quelle, F.W., Nosaka, T., Vignali, D.A., et al. (1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature *380*, 630–633.

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

Spence, R.D., Wisdom, A.J., Cao, Y., Hill, H.M., Mongerson, C.R.L., Stapornkul, B., Itoh, N., Sofroniew, M.V., and Voskuhl, R.R. (2013). Estrogen mediates neuroprotection and anti-inflammatory effects during EAE through ERα signaling on astrocytes but not through ERβ signaling on astrocytes or neurons. J. Neurosci. Off. J. Soc. Neurosci. *33*, 10924–10933.

Spits, H., Artis, D., Colonna, M., Diefenbach, A., Santo, J.P.D., Eberl, G., Koyasu, S., Locksley,

R.M., McKenzie, A.N.J., Mebius, R.E., et al. (2013). Innate lymphoid cells — a proposal for uniform nomenclature. Nat. Rev. Immunol. *13*, 145–149.

Spitzer, M.H., and Nolan, G.P. (2016). Mass Cytometry: Single Cells, Many Features. Cell *165*, 780–791.

Steinert, P.M., and Roop, D.R. (1988). Molecular and cellular biology of intermediate filaments. Annu. Rev. Biochem. *57*, 593–625.

Steinman, R.M. (1991). The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. *9*, 271–296.

Stone, K.D., Prussin, C., and Metcalfe, D.D. (2010). IgE, Mast Cells, Basophils, and Eosinophils. J. Allergy Clin. Immunol. *125*, S73–S80.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Straub, R.H. (2007). The complex role of estrogens in inflammation. Endocr. Rev. *28*, 521–574.

Svotelis, A., Bianco, S., Madore, J., Huppé, G., Nordell-Markovits, A., Mes-Masson, A.-M., and Gévry, N. (2011). H3K27 demethylation by JMJD3 at a poised enhancer of anti-apoptotic gene BCL2 determines ERα ligand dependency. EMBO J. *30*, 3947–3961.

Swain, S.L., Weinberg, A.D., English, M., and Huston, G. (1990). IL-4 directs the development of Th2-like helper effectors. J. Immunol. Baltim. Md 1950 *145*, 3796–3806.

Szabo, S.J., Dighe, A.S., Gubler, U., and Murphy, K.M. (1997). Regulation of the interleukin (IL)-12R beta 2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J. Exp. Med. *185*, 817–824.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A Novel Transcription Factor, T-bet, Directs Th1 Lineage Commitment. Cell *100*, 655–669.

Szego, C.M., and Davis, J.S. (1967). Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen. Proc. Natl. Acad. Sci. U. S. A. *58*, 1711–1718.

Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science *324*, 930–935.

Takeda, K., Tanaka, T., Shi, W., Matsumoto, M., Minami, M., Kashiwamura, S., Nakanishi, K., Yoshida, N., Kishimoto, T., and Akira, S. (1996). Essential role of Stat6 in IL-4 signalling. Nature *380*, 627–630.

Takimoto, T., Wakabayashi, Y., Sekiya, T., Inoue, N., Morita, R., Ichiyama, K., Takahashi, R., Asakawa, M., Muto, G., Mori, T., et al. (2010). Smad2 and Smad3 are redundantly essential for the TGF-beta-mediated regulation of regulatory T plasticity and Th1 development. J. Immunol. Baltim. Md 1950 *185*, 842–855.

Thiede, B., Siejak, F., Dimmler, C., Jungblut, P.R., and Rudel, T. (2000). A two dimensional electrophoresis database of a human Jurkat T-cell line. Electrophoresis *21*, 2713–2720.

Thieu, V.T., Yu, Q., Chang, H.-C., Yeh, N., Nguyen, E.T., Sehra, S., and Kaplan, M.H. (2008). Stat4 is required for T-bet to promote IL-12-dependent Th1 fate determination. Immunity *29*, 679–690.

Thome, M., Charton, J.E., Pelzer, C., and Hailfinger, S. (2010). Antigen receptor signaling to NF-kappaB via CARMA1, BCL10, and MALT1. Cold Spring Harb. Perspect. Biol. *2*, a003004.

Toby, T.K., Fornelli, L., and Kelleher, N.L. (2016). Progress in Top-Down Proteomics and the Analysis of Proteoforms. Annu. Rev. Anal. Chem. *9*, 499–519.

Tsagaratou, A., González-Avalos, E., Rautio, S., Scott-Browne, J.P., Togher, S., Pastor, W.A., Rothenberg, E.V., Chavez, L., Lähdesmäki, H., and Rao, A. (2017). TET proteins regulate the lineage specification and TCR-mediated expansion of *i*NKT cells. Nat. Immunol. *18*, 45–53.

Tsuru, A., Nakamura, N., Takayama, E., Suzuki, Y., Hirayoshi, K., and Nagata, K. (1990). Regulation of the expression of vimentin gene

during the differentiation of mouse myeloid leukemia cells. J. Cell Biol. *110*, 1655–1664.

Tuomela, S., Rautio, S., Ahlfors, H., Öling, V., Salo, V., Ullah, U., Chen, Z., Hämälistö, S., Tripathi, S.K., Äijö, T., et al. (2016). Comparative analysis of human and mouse transcriptomes of Th17 cell priming. Oncotarget *7*, 13416–13428.

Tyanova, S., Temu, T., and Cox, J. (2016). The MaxQuant computational platform for mass spectrometry-based shotgun proteomics. Nat. Protoc. *11*, 2301–2319.

Tzivion, G., Luo, Z.J., and Avruch, J. (2000). Calyculin A-induced vimentin phosphorylation sequesters 14-3-3 and displaces other 14-3-3 partners in vivo. J. Biol. Chem. *275*, 29772–29778.

Urb, M., and Sheppard, D.C. (2012). The Role of Mast Cells in the Defence against Pathogens. PLoS Pathog. *8*.

Vairo, G., Soos, T.J., Upton, T.M., Zalvide, J., DeCaprio, J.A., Ewen, M.E., Koff, A., and Adams, J.M. (2000). Bcl-2 Retards Cell Cycle Entry through p27Kip1, pRB Relative p130, and Altered E2F Regulation. Mol. Cell. Biol. *20*, 4745–4753.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

Venkatesh, J., Yoshifuji, H., Kawabata, D., Chinnasamy, P., Stanevsky, A., Grimaldi, C.M., Cohen-Solal, J., and Diamond, B. (2011). Antigen is required for maturation and activation of pathogenic anti-DNA antibodies and systemic inflammation. J. Immunol. Baltim. Md 1950 *186*, 5304–5312.

Verdú, E.F., Deng, Y., Bercik, P., and Collins, S.M. (2002). Modulatory effects of estrogen in two murine models of experimental colitis. Am. J. Physiol. Gastrointest. Liver Physiol. *283*, G27-36.

Virtakoivu, R., Mai, A., Mattila, E., De Franceschi, N., Imanishi, S.Y., Corthals, G., Kaukonen, R., Saari, M., Cheng, F., Torvaldson, E., et al. (2015). Vimentin-ERK Signaling Uncouples Slug Gene Regulatory Function. Cancer Res. *75*, 2349–2362.

Volpe, E., Servant, N., Zollinger, R., Bogiatzi, S.I., Hupé, P., Barillot, E., and Soumelis, V. (2008). A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. Nat. Immunol. *9*, 650–657.

Wang, Y.-H., Voo, K.S., Liu, B., Chen, C.-Y., Uygungil, B., Spoede, W., Bernstein, J.A., Huston, D.P., and Liu, Y.-J. (2010). A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. J. Exp. Med. *207*, 2479–2491.

Wardenburg, J.B., Fu, C., Jackman, J.K., Flotow, H., Wilkinson, S.E., Williams, D.H., Johnson, R., Kong, G., Chan, A.C., and Findell, P.R. (1996). Phosphorylation of SLP-76 by the ZAP-70 Protein-tyrosine Kinase Is Required for T-cell Receptor Function. J. Biol. Chem. *271*, 19641–19644.

Weckerle, C.E., and Niewold, T.B. (2011). The Unexplained Female Predominance of Systemic Lupus Erythematosus: Clues from Genetic and Cytokine Studies. Clin. Rev. Allergy Immunol. *40*, 42–49.

Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., Cui, K., Kanno, Y., Roh, T.-Y., Watford, W.T., et al. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity *30*, 155–167.

Wirtz, S., Neufert, C., Weigmann, B., and Neurath, M.F. (2007). Chemically induced mouse models of intestinal inflammation. Nat. Protoc. *2*, 541–546.

Worbs, T., Hammerschmidt, S.I., and Förster, R. (2017). Dendritic cell migration in health and disease. Nat. Rev. Immunol. *17*, 30–48.

Wu, J., and Lanier, L.L. (2003). Natural killer cells and cancer. Adv. Cancer Res. *90*, 127–156.

Xiao, B.-G., Liu, X., and Link, H. (2004). Antigen-specific T cell functions are suppressed over the

estrogen-dendritic cell-indoleamine 2,3-dioxygenase axis. Steroids *69*, 653–659.

Xu, H., Liu, J., Cui, X., Zuo, Y., Zhang, Z., Li, Y., Tao, R., Li, Y., and Pang, J. (2015). Increased frequency of circulating follicular helper T cells in lupus patients is associated with autoantibody production in a CD40L-dependent manner. Cell. Immunol. *295*, 46–51.

Xu, L., Kitani, A., Fuss, I., and Strober, W. (2007). Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. J. Immunol. Baltim. Md 1950 *178*, 6725–6729.

Xu, L., Kitani, A., Stuelten, C., McGrady, G., Fuss, I., and Strober, W. (2010). Positive and Negative Transcriptional Regulation of the Foxp3 Gene is Mediated by Access and Binding of the Smad3 Protein to Enhancer I. Immunity *33*, 313–325.

Yamane, H., and Paul, W.E. (2013). Early signaling events that underlie fate decisions of naive CD4(+) T cells toward distinct T-helper cell subsets. Immunol. Rev. *252*, 12–23.

Yang, K., Shrestha, S., Zeng, H., Karmaus, P.W.F., Neale, G., Vogel, P., Guertin, D.A., Lamb, R.F., and Chi, H. (2013). T Cell Exit from Quiescence and Differentiation into Th2 Cells Depend on Raptor-mTORC1-Mediated Metabolic Reprogramming. Immunity *39*, 1043–1056.

Yang, L., Anderson, D.E., Baecher-Allan, C., Hastings, W.D., Bettelli, E., Oukka, M., Kuchroo, V.K., and Hafler, D.A. (2008). IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature *454*, 350–352.

Yang, R., Qu, C., Zhou, Y., Konkel, J.E., Shi, S., Liu, Y., Chen, C., Liu, S., Liu, D., Chen, Y., et al. (2015). Hydrogen Sulfide Promotes Tet1- and Tet2-Mediated Foxp3 Demethylation to Drive Regulatory T Cell Differentiation and Maintain Immune Homeostasis. Immunity *43*, 251–263.

Yang, X.O., Panopoulos, A.D., Nurieva, R., Chang, S.H., Wang, D., Watowich, S.S., and Dong, C. (2007). STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J. Biol. Chem. *282*, 9358–9363.

Ylikomi, T., Wurtz, J.M., Syvälä, H., Passinen, S., Pekki, A., Haverinen, M., Bläuer, M., Tuohimaa, P., and Gronemeyer, H. (1998). Reappraisal of the role of heat shock proteins as regulators of steroid receptor activity. Crit. Rev. Biochem. Mol. Biol. *33*, 437–466.

Yu, D., Rao, S., Tsai, L.M., Lee, S.K., He, Y., Sutcliffe, E.L., Srivastava, M., Linterman, M., Zheng, L., Simpson, N., et al. (2009). The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity *31*, 457–468.

Zhang, W., Sloan-Lancaster, J., Kitchen, J., Trible, R.P., and Samelson, L.E. (1998). LAT: The ZAP-70 Tyrosine Kinase Substrate that Links T Cell Receptor to Cellular Activation. Cell *92*, 83–92.

Zhang, Y., Fonslow, B.R., Shan, B., Baek, M.-C., and Yates, J.R. (2013). Protein Analysis by Shotgun/Bottom-up Proteomics. Chem. Rev. *113*, 2343–2394.

Zhao, D., Long, X.-D., Lu, T.-F., Wang, T., Zhang, W.-W., Liu, Y.-X., Cui, X.-L., Dai, H.-J., Xue, F., and Xia, Q. (2015). Metformin decreases IL-22 secretion to suppress tumor growth in an orthotopic mouse model of hepatocellular carcinoma. Int. J. Cancer *136*, 2556–2565.

Zheng, W., and Flavell, R.A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell *89*, 587–596.

Zheng, S.G., Gray, J.D., Ohtsuka, K., Yamagiwa, S., and Horwitz, D.A. (2002). Generation ex vivo of TGF-beta-producing regulatory T cells from CD4+CD25- precursors. J. Immunol. Baltim. Md 1950 *169*, 4183–4189.

Zhou, L., Ivanov, I.I., Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat. Immunol. *8*, 967–974.

Zhou, L., Chong, M.M.W., and Littman, D.R. (2009). Plasticity of CD4+ T cell lineage differentiation. Immunity *30*, 646–655.

Zhou, X., Kong, N., Wang, J., Fan, H., Zou, H., Horwitz, D., Brand, D., Liu, Z., and Zheng, S.G.

(2010). Cutting edge: all-trans retinoic acid sustains the stability and function of natural regulatory T cells in an inflammatory milieu. J. Immunol. Baltim. Md 1950 *185*, 2675–2679.

Zhu, J., Min, B., Hu-Li, J., Watson, C.J., Grinberg, A., Wang, Q., Killeen, N., Urban, J.F., Guo, L., and Paul, W.E. (2004). Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. Nat. Immunol. *5*, 1157–1165.

Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of effector CD4 T cell populations (*). Annu. Rev. Immunol. *28*, 445–489.

Zhu, J., Jankovic, D., Oler, A.J., Wei, G., Sharma, S., Hu, G., Guo, L., Yagi, R., Yamane, H., Punkosdy, G., et al. (2012). The transcription factor T-bet is induced by multiple pathways and prevents an endogenous T helper-2 program during T helper-1 responses. Immunity *37*, 660–673.

Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2018

ISBN 978-951-29-7394-1 (PRINT)
ISBN 978-951-29-7395-8 (PDF)
ISSN 0355-9483 (PRINT) | ISSN 2343-3213 (PDF)